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Temporal changes in fluoride concentrations in salivary compartments after dentifrice use and professional fluoride applications: Findings from a randomized clinical trial

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ABSTRACT

Background: This study evaluated temporal changes in fluoride concentrations in distinct salivary compartments, supernatant and sediment, up to 12 h after application of fluoridated products with different concentrations and delivery modes.

Methods: This short-term, parallel, randomized clinical trial included 40 participants allocated into four groups: dentifrice containing 1450 ppm F, dentifrice containing 5000 ppm F, acidulated phosphate fluoride (APF) gel, and fluoride varnish. Unstimulated saliva was collected at baseline and at 5, 30, and 60 min, and 4, 8, and 12 h after product application. TISAB-extractable fluoride concentrations were determined in salivary supernatant and sediment and compared using ANOVA, paired t-tests, and ANOVA followed by Dunnett's post hoc test.

Results: For APF gel and fluoride varnish, no significant differences in measured fluoride concentrations were observed between salivary compartments. In contrast, for the 1450 and 5000 ppm F dentifrices, fluoride concentrations were consistently higher in the sediment at all time points. In the supernatant, treatments differed significantly at 5 and 30 min ($p < 0.05$), and from 1 to 8 h both dentifrices showed lower fluoride concentrations than APF gel and varnish. In the sediment, fluoride varnish resulted in higher fluoride concentrations than the other treatments ($p < 0.05$), except at 5 min.

Conclusions: fluoride concentrations in saliva varied according to salivary compartment, time after application, and product formulation. The sediment fraction retained higher fluoride concentrations under several experimental conditions, suggesting a role for the particulate phase in salivary fluoride retention. These findings provide new insights into fluoride kinetics and compartmental distribution in saliva, contributing to the understanding of fluoride behavior as a trace element in biological fluids.

1. Introduction

Fluoride is a biologically active trace element whose presence in body fluids reflects both exposure and local bioavailability [1]. In the oral environment, fluoride is one of the most important agents for caries prevention because it modulates mineral dynamics at the tooth–biofilm interface. Its anticaries effect is mainly related to the reduction of enamel demineralization and the enhancement of remineralization during pH fluctuations caused by biofilm metabolism [2]. Its biological activity depends on its chemical form, as fluoride may be present in saliva as free ions, which are readily bioavailable, or bound to organic and inorganic components, reducing its immediate activity [3]. Saliva is a complex biological fluid that plays a key role in the distribution and clearance of trace elements in the oral cavity [4]. Fluoride bioavailability in saliva is influenced by multiple factors, including product formulation, concentration, and salivary flow rate, and typically shows a rapid increase after exposure, followed by a gradual decline over time [5,6].

Whole saliva can be separated by centrifugation into two distinct compartments: a supernatant and a sediment fraction [7]. The supernatant consists predominantly of water and electrolytes and is essentially free of cells, whereas the sediment contains most of the oral

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microbiome, along with cellular debris, proteins, and food remnants [8–10]. Traditionally, investigations of salivary fluoride bioavailability have focused almost exclusively on the supernatant, demonstrating a rapid post-application increase followed by a progressive decline toward baseline levels within a few hours [6,11]. However, emerging evidence indicates that fluoride concentrations are consistently higher in the salivary sediment than in the supernatant, supporting the concept that this fraction functions as a biological fluoride reservoir capable of sustaining fluoride availability over time [10,12].

Despite this, the compartmental distribution and temporal changes in fluoride concentrations in saliva remain poorly understood, particularly after exposure to fluoride products with different concentrations and delivery systems. Therefore, the present study aimed to evaluate fluoride concentrations in salivary supernatant and sediment up to 12 h after application of fluoridated products. The null hypothesis was that no differences in fluoride concentration would be observed between salivary compartments.

2. Materials and methods

2.1. Ethical aspects

This study was approved by the Research Ethics Committee of the Federal University of Piauí, Brazil (approval numbers 483913 and 543818). All participants signed an informed consent form in accordance with Resolution No. 466 of the National Health Council (Ministry of Health, Brasília, DF, 12/12/2012) and received written and verbal information about the experimental procedures. The study adopted the CONSORT 2025 as a reporting framework and was registered in the Brazilian Clinical Trials Registry (REBEC) under the identifiers RBR−8974jb6 and RBR−10bhvcr4. No deviations or amendments occurred between the registered protocol and the final manuscript.

2.2. Experimental design

This was a short-term, parallel, randomized clinical trial involving 40 adult participants aged 20–35 years. Participants were randomly allocated into four groups according to the fluoride treatment received: Dentifrice containing 1450 ppm F; Dentifrice containing 5000 ppm F; Acidulated phosphate fluoride (APF) gel containing 12,300 ppm F; and Fluoride varnish (Duraphat®, Colgate, Germany) containing 22,600 ppm F. All products were applied according to the manufacturer's instructions. Before the experimental phase, participants used a non-fluoridated dentifrice for three days (lead-in period) [6,13]. The experimental flowchart is presented in Fig. 1, and Table 1 lists the commercial products used, including fluoride concentration, batch number, active ingredient, and other ingredients.

2.3. Pilot study

A pilot study involving six volunteers was conducted to determine sampling time points after product application, to calculate sample size, and to standardize collection and methodological procedures. Those participants were not included in the main trial.

2.4. Recruitment

Participants were undergraduate or graduate dental students from the Federal University of Piauí. Those who agreed to participate were screened according to the inclusion criteria: good oral hygiene, absence of active carious lesions, and normal salivary flow rate. Unstimulated saliva was collected using a graduated tube; individuals able to collect at least 2 mL of saliva within two minutes were included. Exclusion criteria comprised: use of medications that alter salivary flow, use of fluoride supplements, smoking, periodontal disease, fixed orthodontic appliances, allergy or sensitivity to any product ingredients, or professional fluoride application within the past three months. Before the experimental procedures, all participants were clinically examined to confirm eligibility and salivary flow rate. All volunteers were residents of the same city, supplied with fluoridated drinking water, and habitually brushed their teeth twice daily with fluoride dentifrice. Sample size was calculated based on pilot data, considering: four experimental groups; estimated standard deviation = 1.33; minimum detectable difference = 0.85; $\alpha = 5\%$; $\beta = 20\%$. The required total sample was 40 participants. Calculations were performed using G*Power software, version 3.1.9.7 (Heinrich Heine University, Düsseldorf, Germany).

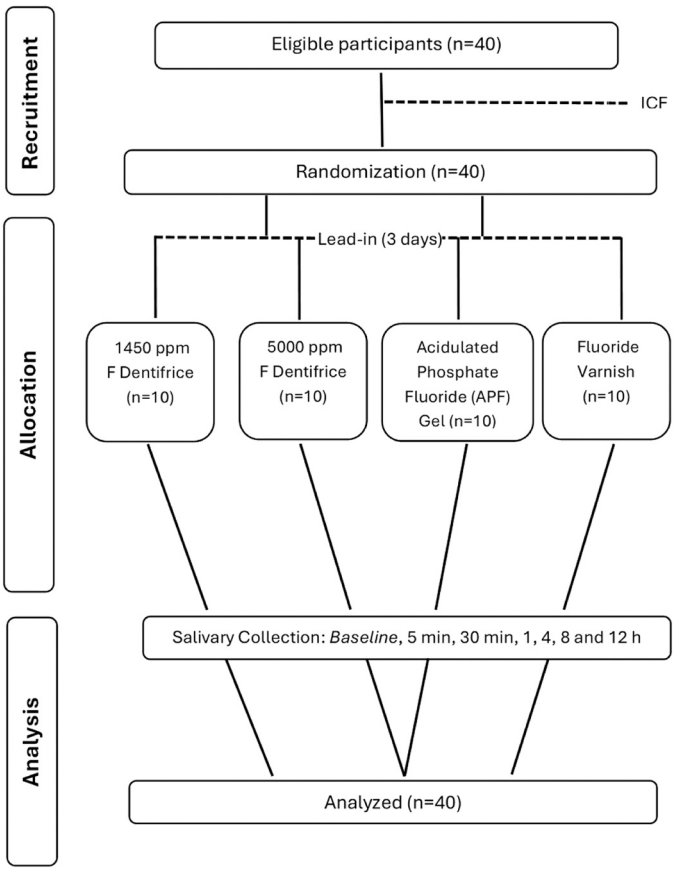

Fig. 1. Flowchart of the experimental design of the study.

2.5. Product application

Application of the fluoride varnish and APF gel was performed by a trained operator for each group, according to the manufacturers' instructions, at the university dental clinic. Prior to product application, all participants underwent dental prophylaxis using pumice slurry (SS White, Rio de Janeiro, Brazil) and a Robinson brush (American Burrs, Santa Catarina, Brazil). For fluoride varnish application, relative isolation was performed and the teeth were dried with compressed air from a three-way syringe. Subsequently, the varnish was applied to the buccal surfaces of all teeth using a microbrush (KG Korense, São Paulo, Brazil), with a total volume of 2 mL, and gently spread over the dental surfaces for 2 min [14]. For APF gel application, a similar protocol was followed, including relative isolation and air-drying of the teeth. Approximately 2 mL of gel was applied to all teeth using a cotton swab and distributed over the dental surfaces for 2 min. After varnish or APF application, participants were allowed to expectorate once immediately to remove excess material.

Toothpaste use was performed directly by the participants after standardized instructions. Toothbrushing was performed using a standardized adult soft-bristle toothbrush provided by the investigators.

Table 1
Description, fluoride content, and ingredients of the products.

Product	Fluoride Concentration (ppm F) reported*	Batch (expiration date)	Active ingredient*	Other ingredients*
Duraphat Varnish Colgate®- Palmolive	22600	012107 (12/26) 012103 (11/26)	NaF	Resin, ethyl alcohol, shellac, mystic, saccharin, flavoring, white beeswax.
Acidulated Phosphate Fluoide Gel (APF)-DFL®	12300	002022 (08/26)	NaF	Sodium Fluoride (1.23% in solution), Phosphoric acid (1.23% in solution), Hydrofluoric acid (0.4% in solution).
Dentifrice with high concentration - OrthoGard- Colgate® - Palmolive	5000	2025900510 (06/26) 2025900510 (06/26)	NaF	Sodium Fluoride (5000 ppm Fluoride), Hydrated Silica, Aroma, Sodium Lauryl Sulfate.
Conventional dentifrice -Colgate® total12	1450	0316AR1123 (03/26) 0316AR113 (05/26)	NaF	Sodium Fluoride (1450 ppm Fluoride), Triclosan 0.3%, Sodium Sulfate, Sorbitol; Hydrated Silica.

* Informed by the manufacturer. NaF = Sodium Fluoride

Participants used an amount of dentifrice equivalent to the length of the toothbrush bristles, corresponding to approximately 1 g. Brushing was performed for 1 min using the modified Bass technique. After expectorating the foam, participants rinsed once with 50 mL of tap water for 10 s [6,15]. All volunteers received a non-fluoridated toothpaste (Condor Baby®, Condor, SC, Brazil) for use during the 3 days preceding the experimental phase, corresponding to the lead-in period.

Participants were instructed to refrain from eating or drinking for the first 2 h after product application and to avoid toothbrushing during the subsequent 12-hour period, corresponding to the final saliva collection time. Additional instructions included avoiding fluoride-containing foods, such as sardines and green tea, and abstaining from eating or drinking (except water) for 1 h prior to each saliva collection. A randomization table was generated using a random number generator (Microsoft Excel, 2016) to determine the order of product application for each participant within each group.

2.6. Saliva sample collection

Unstimulated saliva was collected by expectoration at baseline (immediately before fluoride product application) and at 5 min, 30 min, 1 h, 4 h, 8 h, and 12 h after product application. At each collection time, participants expectorated saliva into graduated plastic containers for up to 3 min, yielding approximately 2 mL per sample. After collection, samples were immediately stored at −20 °C until analysis. Containers were pre-labeled according to treatment and collection time to facilitate sample organization. Participants were also provided with a collection schedule indicating all saliva collection times [6,12].

2.7. Determination of salivary fluoride concentration

Fluoride concentration in saliva was determined using a fluoride-specific electrode (Analyser model 18AF−001, Analyser Instrumentação Analítica, São Paulo, Brazil) coupled to an ion analyzer (Orion EA−740). After thawing, 1 mL aliquots of saliva were homogenized, transferred to microcentrifuge tubes, and centrifuged for 3 min. For supernatant analysis, 0.5 mL of saliva supernatant was buffered with 0.5 mL of TISAB II. The remaining supernatant was discarded, leaving only the solid fraction. Subsequently, 0.5 mL of TISAB II was added to the sediment and vortexed for 10 s using a vortex mixer (Nova Instrumentos, Piracicaba, SP, Brazil) [10,12]. The resulting suspension was then analyzed to determine fluoride concentration. Fluoride concentrations in both salivary compartments were calculated by linear regression from a calibration curve generated using fluoride standards ranging from 0.125 to 32 μg F/mL, prepared under the same conditions as the samples [6]. The fluoride-specific electrode showed a mean slope of −58 mV/decade during calibration, which was within the expected range for fluoride ion-selective electrode analysis. In this study, TISAB II was added to all samples before fluoride measurement to standardize ionic strength and pH, reduce interference from polyvalent ions, and allow determination of total recoverable fluoride under controlled analytical conditions. Therefore, the fluoride concentrations reported represent TISAB-extractable fluoride rather than exclusively free ionic fluoride present in saliva under physiological conditions.

2.8. Area under the curve and ratio supernatant/sediment calculation

The cumulative fluoride exposure in each salivary compartment was estimated by calculating the area under the curve (AUC). AUC values were obtained separately for the salivary supernatant and sediment using GraphPad Prism software, version 9.51 (GraphPad Software, La Jolla, CA, USA). The analysis was performed using the trapezoidal rule, in which the area between two consecutive sampling points is calculated from the mean fluoride concentrations observed at those time points and the corresponding time interval. The sum of these partial areas represented the total fluoride exposure during the 12-hour observation period. The actual collection times used for the calculation were baseline/pre-application, 5, 30, 60, 240, 480, and 720 min after fluoride product application.

The sediment/supernatant ratio was calculated for each treatment and collection time by dividing the overall mean fluoride concentration obtained in the sediment by the overall mean fluoride concentration obtained in the supernatant.

2.9. Statistical analysis

Data normality was assessed using the Shapiro–Wilk test, which indicated a non-normal distribution. Therefore, data were log10-transformed prior to statistical analysis. Differences among treatments within each salivary compartment were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Paired *t*-tests were applied to compare fluoride concentrations between supernatant and sediment for each treatment at each collection time. Comparisons between post-application fluoride levels and baseline values within each treatment were performed using ANOVA followed by Dunnett's multiple comparison test. Descriptive statistical data were presented as tables and figures. All analyses were conducted using GraphPad Prism software (version 9.51; GraphPad Software, La Jolla, CA, USA).

3. Results

Table 2 summarizes TISAB-extractable fluoride concentrations (μg F/

Table 2
Mean ±SD (n = 10) of fluoride concentration in the salivary supernatant or sediment (μg F/mL) according to the fluoride products used.

	Supernatant				Sediment			
	Dent. 1450	Dent. 5000	APF	Varnish	Dent. 1450	Dent. 5000	APF	Varnish
Baseline	0.05 ± 0.02 aA	0.06 ± 0.04 aA	0.07 ± 0.03 aA	0.04 ± 0.01 aA	0.18 ± 0.09 bA	0.16 ± 0.04 bA	0.16 ± 0.17 bA	0.15 ± 0.04 bA
5 min	1.36 ± 0.51 aA	4.13 ± 2.80 aB	54.27 ± 35.22 aC	113.85 ± 103.03 aD	0.88 ± 0.39 bA	5.85 ± 2.60 bB	36.99 ± 23.09 aC	35.8 ± 13.64 aC
30 min	0.18 ± 0.08 aA	0.62 ± 0.52 aB	4.55 ± 2.58 aC	22.01 ± 10.12 aD	0.33 ± 0.17 bA	1.41 ± 1.01 bB	7.01 ± 6.45 aC	11.79 ± 4.36 aD
1 h	0.10 ± 0.04 aA	0.21 ± 0.16 aA	1.79 ± 0.90 aB	14.38 ± 8.39 aC	0.27 ± 0.15 bA	0.65 ± 0.39 bB	3.94 ± 4.13 aC	10.68 ± 3.86 aD
4 h	0.05 ± 0.02 aA	0.07 ± 0.03 aA	0.29 ± 0.11 aB	2.53 ± 2.07 aC	0.19 ± 0.10 bA	0.40 ± 0.24 bAB	0.60 ± 0.48 a.B	5.19 ± 3.57 aC
8 h	0.04 ± 0.01 aA	0.07 ± 0.07 aA	0.14 ± 0.05 aB	0.95 ± 1.04 aC	0.17 ± 0.07 bA	0.19 ± 0.06 bA	0.34 ± 0.21 aA	2.39 ± 1.99 aB
12 h	0.03 ± 0.01 aA	0.05 ± 0.04 aAB	0.08 ± 0.01 aBC	0.27 ± 0.34 aC	0.21 ± 0.13 bA	0.22 ± 0.09 bA	0.15 ± 0.07 aA	0.92 ± 0.69 aB

Lowercase letters denote significant difference between the supernatant and the sediment within the same product and uppercase letters denote significant difference between the products within each salivary compartment (supernatant or sediment), $p < 0.05$.

mL) measured in the salivary supernatant and sediment following the use of the different fluoride products at each observation period. No significant differences among treatments were observed at baseline for either compartment ($p > 0.05$). However, within each treatment, baseline fluoride concentrations were significantly higher in the sediment than in the supernatant ($p < 0.05$). For the higher-fluoride products (APF gel and varnish), this compartmental difference was limited to baseline, whereas for the 1450 and 5000 ppm F dentifrices, measured fluoride concentrations remained consistently higher in the sediment at all time points ($p < 0.05$).

In the salivary supernatant, treatments differed significantly at 5 and 30 min post-application ($p < 0.05$). From 1–8 h, both dentifrices showed lower fluoride concentrations than APF gel and varnish ($p < 0.05$), with no differences between the two dentifrices ($p > 0.05$). Fluoride varnish produced the highest supernatant fluoride concentrations at all time points ($p < 0.05$), except at 12 h, when values did not differ from those observed for APF gel ($p > 0.05$). A similar pattern was observed in the salivary sediment, in which varnish resulted in higher measured fluoride concentrations than all other treatments ($p < 0.05$), except at 5 min, when it did not differ from APF gel ($p > 0.05$).

Table 3 presents the sediment/supernatant ratio calculated from mean fluoride concentrations. At baseline, the ratio was greater than 1 for all treatments, showing that measured fluoride concentrations were higher in the sediment before experimental product application. At 5 min, the ratio decreased below 1 for the 1450 ppm F dentifrice, APF gel, and varnish groups, reflecting higher mean fluoride concentrations in the supernatant immediately after exposure. In contrast, the 5000 ppm F dentifrice maintained a ratio above 1 at this time point. From 30 min onward, ratios remained above 1 for both dentifrice groups and for APF gel. For fluoride varnish, the ratio remained below 1 up to 1 h and exceeded 1 only from 4 h onward, showing a delayed shift toward higher mean fluoride concentrations in the sediment.

The area under the curve (AUC) analysis (Fig. 2) showed that cumulative measured fluoride concentrations over time increased according to product fluoride concentration, independent of the salivary compartment, with all treatments differing significantly from one another ($p < 0.05$). AUC values were higher in the sediment only for the dentifrice groups (1450 and 5000 ppm F), reflecting higher measured fluoride concentrations over time in this compartment ($p < 0.05$). No compartmental differences in AUC were observed for APF gel or fluoride varnish ($p > 0.05$).

Table 3
Ratio of the mean values of fluoride concentration in the sediment to the mean values of fluoride in the salivary supernatant in the fluoride products used.

Treatments	Time						
	Baseline	5 min	30 min	1 h	4 h	8 h	12 h
Dent. 1450	3,47	**0,65**	1,80	2,66	3,64	3,84	6,03
Dent. 5000	2,89	1,42	2,27	3,04	5,46	2,76	4,09
APF	2,23	**0,68**	1,54	2,20	2,08	2,43	1,84
Varnish	3,68	**0,31**	**0,54**	**0,74**	2,05	2,53	3,37

Bold values indicate a higher fluoride concentration in the supernatant relative to the sediment.

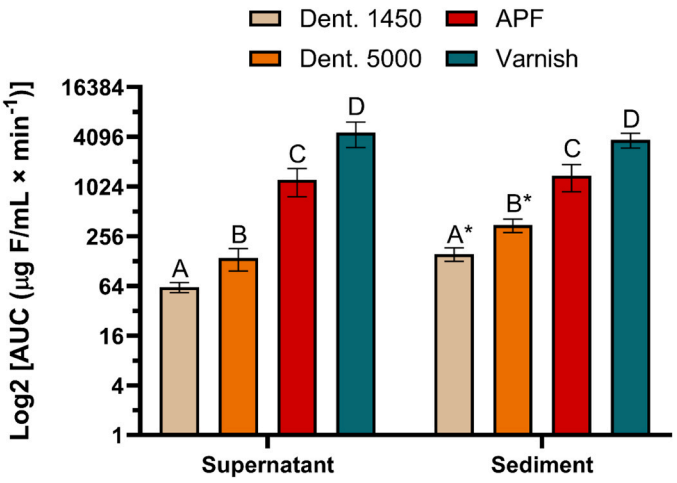

Fig. 2. Mean area under the curve (AUC) of fluoride concentration in salivary supernatant and sediment over time (μg F/mL·min^{-1}) according to the treatments (n = 10). Different letters indicate statistically significant differences among treatments within each salivary compartment, and asterisks indicate statistically significant differences between salivary compartments within each treatment ($p < 0.05$). Vertical bars represent standard deviation, and values are plotted on a $\log_2$ scale to facilitate visualization.

Fig. 3 depicts the temporal concentration profiles of fluoride in both salivary compartments. All treatments produced a significant increase in measured salivary fluoride concentrations, with peak values occurring at 5 min. Thereafter, fluoride concentrations declined for all products. However, fluoride varnish maintained higher measured fluoride concentrations in both compartments throughout the 12-hour observation period. For APF gel, elevated fluoride concentrations persisted up to 8 h in the supernatant and 4 h in the sediment. In contrast, fluoride concentrations returned to baseline within 4 h after brushing for both dentifrices in the supernatant, whereas in the sediment, baseline levels were reached after 4 h for the 5000 ppm F dentifrice and after 1 h for the 1450 ppm F dentifrice ($p < 0.05$).

4. Discussion

Fluoride is a biologically active trace element whose concentration in body fluids reflects recent exposure, local retention, and redistribution dynamics [1]. In saliva, fluoride plays a central role in regulating mineral equilibrium at the tooth–biofilm interface, and its biological effectiveness is strongly dependent on its bioavailable fraction [2]. In this context, the present study expands current knowledge by showing that fluoride behavior in saliva is compartment-dependent and not restricted to the fluid phase. Therefore, the null hypothesis that fluoride

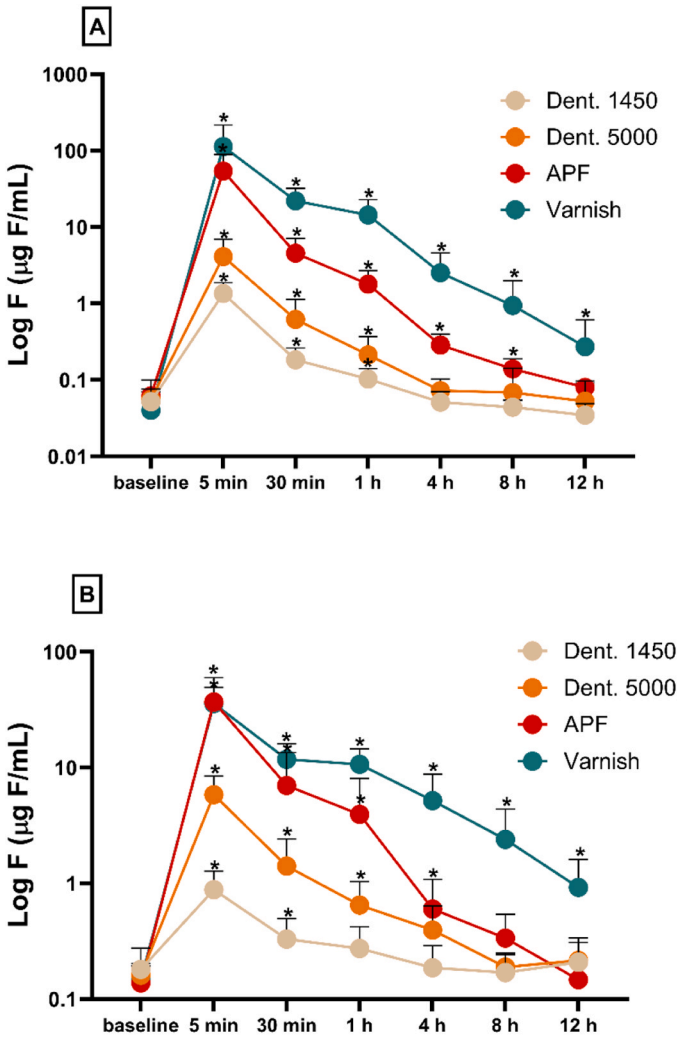

Fig. 3. Temporal profiles of fluoride concentration (mean ± SD, n = 10; µg/mL) in salivary supernatant (A) and sediment (B) after the use of fluoridated products. Asterisks indicate statistically significant differences between each time point and baseline ($p < 0.05$).

concentrations would not differ between salivary compartments after exposure to products with different fluoride concentrations was rejected.

Before interpreting these findings, it is important to consider the analytical nature of the fluoride measurements performed in this study. Because TISAB II was added before fluoride determination, the values obtained represent total recoverable, or TISAB-extractable, fluoride under standardized analytical conditions. This approach allows comparison among treatments and salivary compartments, but it does not distinguish free ionic fluoride in solution from fluoride bound to salivary components or released from acid-labile reservoirs, such as CaF_2-like deposits. Therefore, the results should be interpreted as measured fluoride concentrations in each salivary fraction rather than as a direct estimate of physiologically free or immediately bioactive fluoride.

The higher fluoride concentrations observed in the salivary sediment at baseline reinforce the concept that this compartment functions as an oral fluoride reservoir. This finding agrees with previous studies [10,12] and suggests that fluoride is not homogeneously distributed in whole saliva. Instead, it appears to preferentially associate with the solid fraction, which contains cellular structures, proteins, microorganisms, and organic debris. These components may provide binding sites for fluoride ions, allowing reversible adsorption and subsequent release. Such interactions may help explain why fluoride remains detectable in saliva even in the absence of recent fluoride exposure.

After product application, fluoride concentrations in the supernatant showed the expected pattern of a rapid increase followed by a gradual decline. However, the magnitude and persistence of this response varied markedly among treatments. Dentifrices produced lower and shorter-lived fluoride increases, whereas professional products, especially fluoride varnish, maintained higher concentrations for most of the observation period. These differences are consistent with previous reports [16,17] and are likely related to formulation and delivery mode. Varnishes adhere to dental surfaces, prolong fluoride contact with enamel, and favor the formation of calcium fluoride-like deposits, which may act as sources of sustained fluoride release [16,18]. In the early post-application period, the high fluoride levels observed after varnish use may also reflect residual material retained in the oral cavity due to its viscous and adhesive formulation.

Fluoride distribution in saliva is influenced by several factors, including product concentration, formulation, application method, substantivity, and salivary flow [19–21]. The present findings reinforce this concept by showing that the sediment fraction also responds differently according to product type. Fluoride varnish promoted greater retention in the sediment than the other treatments, suggesting that this compartment contributes substantially to fluoride persistence after professional application. This observation is relevant because many previous studies have focused mainly on salivary supernatant and may therefore have underestimated the role of the particulate fraction in fluoride retention and redistribution.

The sediment/supernatant ratio provides an additional perspective on fluoride partitioning between the liquid and particulate phases of whole saliva. Dentifrice and APF gel groups showed an earlier predominance of fluoride in the sediment, suggesting rapid redistribution from the fluid phase to binding sites present in cellular debris, proteins, microorganisms, and other salivary solids. In contrast, fluoride varnish exhibited a distinct temporal pattern, with ratios below 1 during the first hour and a shift toward sediment predominance only from 4 h onward. This delayed inversion may be explained by the adhesive and viscous nature of the varnish, which initially maintains fluoride-containing material on dental surfaces and gradually releases fluoride into the salivary fluid before subsequent association with the sediment fraction [14].

Interestingly, the early fluoride concentrations in the sediment were comparable between varnish and APF gel, despite their differences in fluoride concentration and delivery mode. This finding may indicate that the salivary sediment has a limited short-term binding capacity. Once available binding sites are saturated, additional fluoride may remain predominantly in the supernatant or be cleared from the oral cavity. From a mechanistic perspective, this suggests that fluoride retention in saliva depends not only on the amount of fluoride delivered, but also on how the formulation interacts with oral surfaces, saliva, and particulate salivary components.

Thus, the formulation-related factors appear to play a central role in the time-dependent behavior and compartmental distribution of fluoride. Dentifrices are rapidly dispersed and cleared after brushing and rinsing, which may explain their lower and shorter fluoride exposure [6]. APF gel and varnish, on the other hand, provide higher fluoride availability and favor the formation of transient oral reservoirs. Among these products, varnish showed the most sustained profile, probably due to its prolonged surface retention and gradual fluoride release [14]. Therefore, the differences observed among treatments should not be interpreted solely as a function of fluoride concentration, but as the result of interactions among concentration, formulation, delivery mode, oral clearance, and compartmental binding.

The temporal profile observed in this study supports a biphasic pattern of fluoride behavior in saliva. The first phase is characterized by a rapid increase in fluoride concentration immediately after product application, mainly reflecting product dissolution and direct release into saliva. This is followed by a slower phase, governed by fluoride

redistribution, binding to salivary sediment, interaction with oral reservoirs, and gradual clearance. This pattern is consistent with previous reports [10] and highlights the importance of evaluating both supernatant and sediment when studying salivary fluoride dynamics.

The area under the curve analysis further supports these interpretations. Fluoride exposure increased according to product fluoride concentration, regardless of salivary compartment [22,23]. However, the higher AUC values observed in the sediment for the dentifrice groups suggest greater relative retention of fluoride in this compartment after products with lower substantivity [17,24]. For APF gel and varnish, no compartmental differences in AUC were detected, possibly because professional formulations promote broader fluoride concentration in both the fluid and particulate phases. This may be related to the formation of calcium fluoride-like reservoirs, which can gradually release fluoride over time [25].

The concentration–time profiles also showed that all products produced a rapid fluoride peak followed by progressive decline [5,22,23]. Although professional applications maintained elevated fluoride levels for longer periods than dentifrices, fluoride concentrations decreased over time and approached baseline values, confirming that these oral reservoirs are transient. Previous studies have similarly shown that salivary fluoride levels may return to baseline after cessation of exposure [26]. These findings reinforce the importance of repeated fluoride delivery, either through daily dentifrice use or periodic professional application, to maintain fluoride availability in oral fluids.

From a clinical perspective, the fluoride concentrations observed should be interpreted with caution. Although sustained low-level fluoride in oral fluids is considered important for reducing enamel demineralization and favoring remineralization during cariogenic challenges [27], the present study did not quantify the fraction of fluoride present as free ions under physiological conditions. Therefore, the clinical relevance of the results should be understood in terms of measured fluoride retention and compartmental distribution, rather than direct bioactive fluoride availability. The 1450 ppm F dentifrice produced a short-lived increase in salivary fluoride, compatible with its role as a routine home-care product. The 5000 ppm F dentifrice resulted in higher measured fluoride concentrations than the conventional dentifrice, supporting its use in individuals at higher caries risk [28]. APF gel and fluoride varnish produced markedly higher values, consistent with their role as professional preventive interventions. Among these, varnish maintained the most sustained concentration profile, suggesting greater substantivity and prolonged retention of fluoride-containing material [29]. Therefore, translation of these findings to oral health should consider not only peak fluoride concentration, but also retention time, compartmental distribution, product formulation, frequency of use, and the analytical limitation that free and bound fluoride forms were not differentiated.

This study has some limitations that should be considered when interpreting the findings. First, fluoride was measured after the addition of TISAB II, and the results should therefore be interpreted as total TISAB-extractable fluoride rather than exclusively free ionic fluoride under physiological salivary conditions. Second, the study described short-term concentration–time profiles and cumulative fluoride exposure but did not apply formal kinetic modeling or estimate specific release constants. Finally, dentifrice use was performed under supervised and standardized conditions, including a fixed brushing time and rinsing protocol, which may not fully reproduce individual home-care behaviors. Future studies should address those limitations.

5. Conclusion

Measured fluoride concentrations in saliva were influenced by salivary compartment, time after application, and formulation-specific properties. The salivary sediment retained higher fluoride concentrations than the supernatant under several experimental conditions, suggesting that the particulate fraction may contribute to fluoride retention in whole saliva. Professional fluoride products, particularly varnish, produced higher and more sustained measured fluoride concentrations, likely because of their concentration, substantivity, and delivery characteristics. These findings highlight the importance of considering both salivary compartments and product formulation when evaluating fluoride distribution in biological fluids. However, as free and bound or reservoir-associated fluoride forms were not differentiated, the clinical relevance of these findings should be interpreted with caution.

Author contribution

E.P. performed the study, analyzed the results, and drafted the manuscript. L.R. and Y.F. performed the fluoride analyses and revised the manuscript. L.S revised the manuscript. G.V. supervised the study, performed the statistical analyses, and wrote the final version of the manuscript.

CRediT authorship contribution statement

Lucas Lopes Araújo Sousa: Writing – original draft, Visualization. **Vale Glauber Campos:** Writing – review & editing, Project administration, Investigation, Formal analysis, Conceptualization. **Lyzia Vitória Mendes Rezende:** Writing – original draft, Methodology. **Yarlla Rayanne Nogueira dos Anjos Franco:** Writing – original draft, Methodology. **Evanildo Canuto Paz:** Writing – original draft, Methodology, Investigation.

Informed consent

Informed consent was obtained from all participants included in the study.

Ethics declaration

This study was approved by the Research Ethics Committee of the Federal University of Piauí, Brazil (approval numbers 483913 and 543818).

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Declaration of Competing Interest

The authors declare no competing interests.

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Data availability

Data will be available on reasonable request.

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