

A simplified protocol to determine total fluoride concentration in NaF/silica-based toothpastes

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Aim: To determine total fluoride (TF) concentration in Na₂FPO₃/Ca-based toothpastes, using fluoride ion selective electrode (F-ISE) by the direct technique, it is necessary to use acid (Ac⁺) to hydrolyze the FPO₃²⁻ ion and to dissolve insoluble fluoride salts bound to the abrasive. For NaF/silica-based toothpastes, the use of acid is not necessary (Ac⁻) and a simplified protocol could be followed. **Methods:** Thus, we evaluated TF concentration in seven brands of NaF/silica-based toothpastes, following the validated conventional Cury's protocol (Ac⁺) or a simplification of this protocol (Ac⁻). Fluoride was analyzed with ISE calibrated with fluoride standard solutions prepared in the same conditions as the samples (Ac⁺ or Ac⁻). **Results:** The mean (±SD; n=21) of TF concentrations (µg F/g) found by Ac⁺ (971.3±191.2) and Ac⁻ (982.4±201.3) protocols were not statistically different (t test, p=0.22). The TF concentrations found agree with those declared by the manufacturers, except for one toothpaste imported from China. **Conclusion:** The findings suggest that the determination of fluoride in NaF/silica-based toothpastes can be accurately made using a simplified protocol of analysis.

Key words: Fluorides. Toothpastes. Silicic acid. Dentifrices. Ion-selective electrodes.



Introduction

Toothpaste is considered the most rational way of fluoride use to control caries and the total fluoride concentration in toothpastes is under legislation control worldwide. Thus, different methods are used to determine fluoride concentration in toothpastes¹ and fluoride ion specific electrode (F-ISE) has been used for a long time^{2,3}. The choice of the method for analysis mainly depends on the type of fluoride salt present in the formulation and the abrasive system of the toothpaste.

In toothpastes containing calcium-based abrasives, such as calcium carbonate (CaCO_3) or calcium phosphate dihydrate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$), sodium monofluorophosphate (Na_2FPO_3) is the source of fluoride salt used due to the chemical incompatibility of sodium fluoride (NaF), stannous fluoride (SnF_2) or amine fluoride (AmF) with calcium (Ca^{++}). In formulations $\text{Na}_2\text{FPO}_3/\text{Ca}$ -based, part of the total fluoride (TF) is soluble (TSF) as PO_3F^{2-} ion plus fluoride ion (F^-), but part is insoluble (Fins.) bound to the abrasive⁴. NaF , SnF_2 and AmF toothpastes are formulated with hydrated silica (SiO_2), in which TF is soluble as F^- . Fluoride ion specific electrode (F-ISE) has been successfully used to determine the different forms of fluoride in $\text{Na}_2\text{FPO}_3/\text{Ca}$ -based and in NaF/SiO_2 -based toothpastes following the same protocol of analysis⁵.

To determine total fluoride in $\text{Na}_2\text{FPO}_3/\text{Ca}$ -based toothpastes with F-ISE, acid is used to hydrolyze FPO_3^{2-} ion and to dissolve insoluble fluoride salts bound to the abrasive⁵. For the determination of TF in Ca-free NaF toothpastes with F-ISE, it is not necessary to use acid because all TF is soluble as F^- to be promptly analyzed with the electrode. Although the use of acid is not theoretically necessary and the protocol used since 1980 by Cury et al. (1981)³ is valid because there is a high correlation ($p=0.996$) between TF found and that present in 27 NaF/silica -based toothpastes⁶, the protocol could be simplified.

Therefore, we evaluated if the step of acid hydrolysis used in the validated protocol of Cury et al. (2010)⁵, could be simplified with accuracy for the determination of TF in NaF/silica -based toothpastes.

Material and Methods

Sampling

Seven brands ($n=3/\text{brand}$) of fluoride toothpastes marketed for children were purchased; six in Lima, Peru, and one in Piracicaba, Brazil. Each tube from each brand was purchased in different supermarkets and drugstores and were from different fabrication batches, except for Peppa Pig brand. Table 1 shows information about the toothpastes used. Complying with the purpose of this study, all toothpastes chosen were NaF/silica -based and were analyzed within their expiration time.

Table 1. Information on the toothpastes analyzed

Brand	Code	Country of Purchase	Abrasive agent	Fluoridated agent	Declared [F] (ppm F)	Expiration date
Colgate 6+	A ₁	Peru	Silica	NaF	1100	01/2020
Colgate 6+	A ₂	Peru	Silica	NaF	1100	02/2020
Colgate 6+	A ₃	Peru	Silica	NaF	1100	10/2019
Aqua Fresh My Big	B ₁	Peru	Silica	NaF	1150	02/2019
Aqua Fresh My Big	B ₂	Peru	Silica	NaF	1150	09/2018
Aqua Fresh My Big	B ₃	Peru	Silica	NaF	1150	06/2018
Vitis Jr	C ₁	Peru	Silica	NaF	1000	05/2019
Vitis Jr	C ₂	Peru	Silica	NaF	1000	12/2019
Vitis Jr	C ₃	Peru	Silica	NaF	1000	04/2019
Colgate Kids	D ₁	Peru	Silica	NaF	1100	02/2020
Colgate Kids	D ₂	Peru	Silica	NaF	1100	02/2020
Colgate Kids	D ₃	Peru	Silica	NaF	1100	02/2020
Dento Jr	E ₁	Peru	Silica	NaF	1100	04/2020
Dento Jr	E ₂	Peru	Silica	NaF	1100	10/2019
Dento Jr	E ₃	Peru	Silica	NaF	1100	-
Peppa Pig	F ₁	Peru	Silica	NaF	1450	06/2021
Peppa Pig	F ₂	Peru	Silica	NaF	1450	-
Peppa Pig	F ₃	Peru	Silica	NaF	1450	-
Tandy	G ₁	Brazil	Silica	NaF	1100	02/2020
Tandy	G ₂	Brazil	Silica	NaF	1100	02/2020
Tandy	G ₃	Brazil	Silica	NaF	1100	02/2020

Toothpaste preparation for fluoride analysis

Toothpastes samples were prepared (Figure 1) for analysis of total fluoride (TF) with F-ISE by the protocol described by Cury et al. (2010)⁵. An amount of 90 to 110 mg of toothpaste was weighed (± 0.01 mg) and vortexed in 10.0 mL of purified water. Duplicate volumes of 0.25 and 1.0 mL of the suspension were transferred to tubes codified Ac⁺ and Ac⁻, respectively conventional (Figure 1A) and simplified protocols (Figure 1B). To the tubes Ac⁺, 0.25 mL of 2.0 M HCl was added and after 1 h at 45 °C the extracts were buffered with 0.50 mL of 1.0 M NaOH plus 1.0 mL of TISAB II. To the tubes Ac⁻, just 1.0 mL of TISAB II was added.

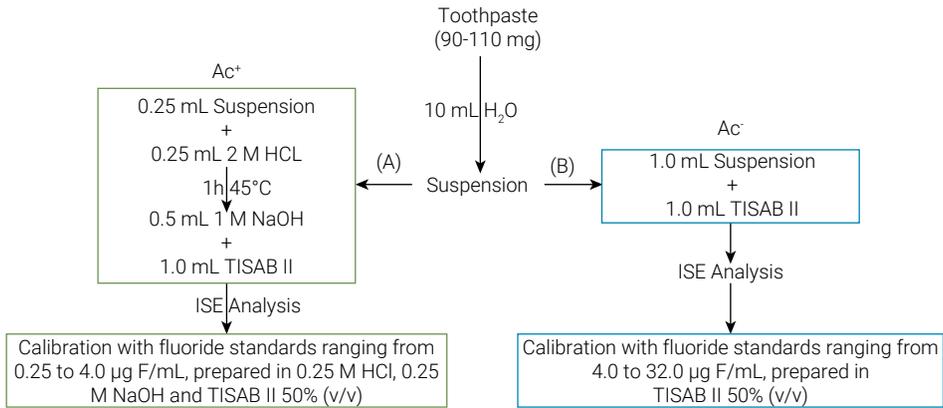


Figure 1. Toothpaste sample preparation for total fluoride determination by the conventional (A) and the simplified protocol (B)

Two calibration curves were made (Figure 2). For samples prepared according to the conventional protocol (Ac⁺; Figure 1A), standards ranging from 0.25 to 4.0 µg F/mL prepared in 0.25 M HCl, 0.25 M NaOH and TISAB II 50% (v/v) were used. For samples evaluated by the simplified protocol (Ac⁻; Figure 1B), fluoride standards ranging from 4.0 to 32.0 µg F/mL prepared in TISAB II 50% (v/v) were used. All fluoride standards were prepared from NaF 99.99% (Sigma-Aldrich, St Louis, MO, USA). The accuracy of the analysis was checked with a standard fluoride solution (Orion 940907, Thermo Scientific, Boston, MA, USA) and the average coefficient of variation from triplicates was 1.0 and 2.4%, respectively for the conventional and the simplified protocol. For the analysis, a F-ISE (Orion 96-06; Orion Research Inc., Boston, MA, USA) coupled to an ion analyzer (Orion Star A214; Orion Research Inc., Boston, MA, USA) was used. Fluoride concentration in the samples was determined from linear regression of the logarithm of fluoride concentrations of the standards with the respective mV values ($r^2=0.999$ for both calibration curves), using Excel spreadsheet (Microsoft®). The results were expressed in ppm F (µg F/g; mg F/kg).

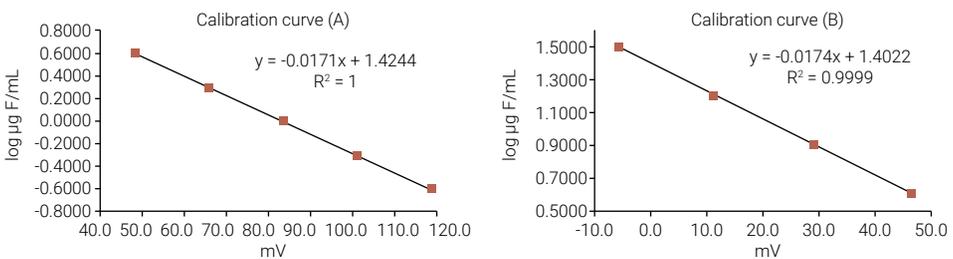


Figure 2. Calibration curves (n=3) and data of the correlation between the logarithm of fluoride concentrations in standards and the relative mV generated for the conventional (A) and simplified (B) protocols of analysis.

Statistical analysis

The data of TF found in the 21 samples, analyzed according to the conventional protocol using acid (Ac⁺) and those found by the simplified protocol (Ac⁻), were compared by paired *t* test at 5%.

Results

Figure 3 shows total fluoride (TF) concentration declared by the manufacturer and the mean (SD; n=3) concentrations found by the conventional protocol (Ac⁺; acid treatment) and by the simplified one (Ac⁻; without acid treatment) of the seven toothpastes evaluated. Excluding the unusual finding for Peppa Pig toothpaste, the difference (%) between TF found and that declared for the other six toothpastes was -4.1 and -2.8%, respectively for the conventional and simplified protocol.

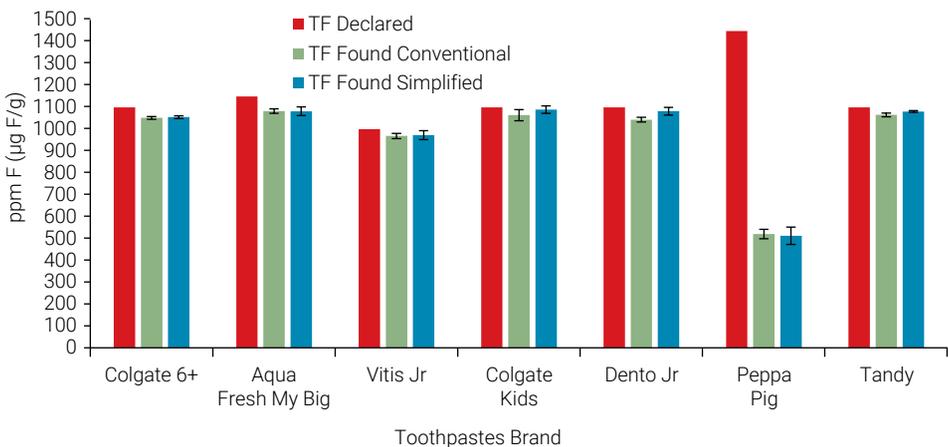


Figure 3. Total fluoride (TF) declared (ppm F) by the manufacturer and concentrations found using the conventional (Ac⁺) and the simplified (Ac⁻) protocol (Mean;SD;n=3)

Figure 4 shows means (SD; n=21) of fluoride concentration found in the toothpaste tubes by the conventional protocol and by the simplified one. The difference between the protocols was not statistically significant ($p=0.22$). The variation coefficient in percentage was 19.8 and 20.5%, respectively by the conventional and simplified protocol.

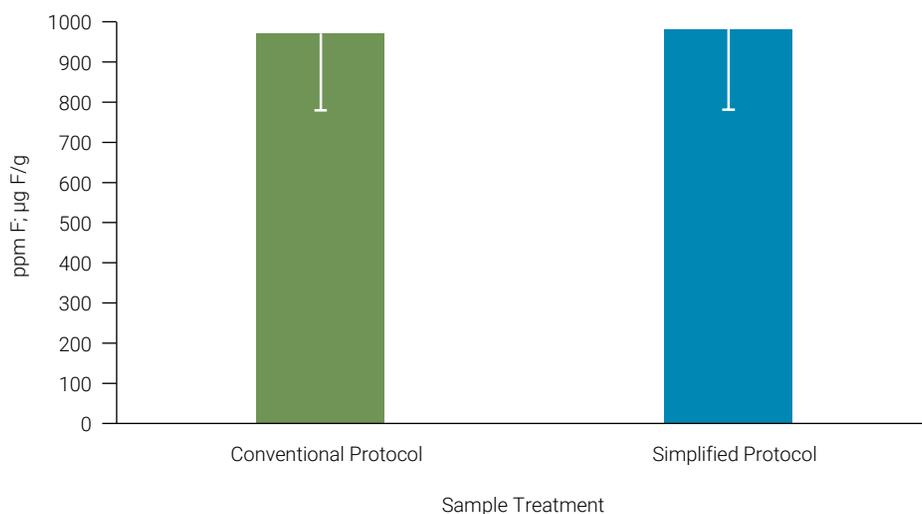


Figure 4. Fluoride concentration (ppm F) found in the toothpastes by the conventional (Ac⁺) and the simplified (Ac⁻) protocol; (Mean;SD;n=7;p=0.22)

Discussion

The protocol of fluoride determination in toothpastes with F-ISE by the direct technique and used since 1980 in Laboratory of Oral Biochemistry from FOP-UNICAMP^{3,5,7-20} is considered chemically valid, reliable, and feasible¹. In addition, it is able to estimate how much of the total fluoride presents in Na₂FPO₃/CaCO₃-based toothpaste is bioavailable to be released in the oral cavity during toothbrushing²¹. However, this protocol could be simplified to determine fluoride in Ca-free toothpaste formulations, which contains ionic fluoride salts, such as NaF.

The findings clearly showed that the simplified protocol used is valid, reliable, and feasible when compared with the results found for the conventional protocol. As shown in figure 4, the mean of total fluoride found in the 21 samples of toothpastes analyzed with the simplified protocol did not statistically differ from the conventional protocol. The results found with the simplified protocol were as reproducible as those found with the conventional protocol, because the variation coefficient (%) of duplicates of analyses (n=21) was 1.3% compared with 1.5% for the conventional.

The simplified protocol is cheaper, consumes less time of work and is easier than the conventional one, because the number of laboratorial steps is reduced from the calibration curve up until the sample determination. Therefore, it can be used instead of the conventional protocol not only for NaF/Ca-free-based toothpastes as well as for SnF₂ or AmF formulations. However, the analyst should check if in fact the commercial toothpaste was not formulated with Na₂FPO₃ or the abrasive is Ca-free. This warning is important because we have previously found toothpastes containing CaCO₃ as abrasive but according to the manufacturer it was silica¹⁹.

In all toothpastes, except in Peppa Pig, TF concentration found agreed with the declared by the manufacturer. According to the label of Peppa Pig's toothpaste, it should contain 1450 ppm F but we found 512.0 and 520.7 ppm F, respectively for the conventional and the simplified protocol. This result confirmed our previous fluoride determination in other batches of this same toothpaste¹⁹, where we found 515.1 ppm F using our conventional protocol⁵.

In addition to the development of this simplified protocol for fluoride determination in toothpastes, the present data confirm the necessity of sanitary vigilance of imported toothpastes from China because if there are approximately 3 times lower TF than the declared in the Peppa Pig brand analyzed, in previous analyses we found the opposite¹⁴. Also, we have observed in the present study problems with the type of abrasive declared and that found, as it is already described by Chávez et al. (2019)¹⁹.

Furthermore, the present study confirms that when the use of acid is necessary to hydrolyze PO_3F^{2-} ion and to dissolve insoluble F bound to abrasive, HCl as used in Cury's protocol (2010)⁵ does not produce artifacts in the analysis because the TF concentration found did not statistically differ comparing the results found with the simplified protocol (Figure 3).

In conclusion, the findings suggest that the determination of fluoride in NaF/silica-based toothpastes can be accurately made using a simplified protocol of analysis.

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