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Method for the Analysis of Total Fluoride in Fluoride-Releasing Dental Varnishes

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Abstract

Today's fluoride-releasing varnishes (F-varnish) contain a wide variety of ingredients which present analytical challenges for measuring their total fluoride content. This study reports improved methods to measure fluoride content in F-varnishes. Six different commercially available F-varnishes that contain difluorosilane (0.1% F) or NaF (2.26% F) alone or in combination with calcium-phosphates were analyzed. In a vial, 1–3 drops (0.05–0.15 g) of varnish product was dispensed, dissolved in chloroform, equilibrated in TISAB and analyzed via fluoride ion-selective electrode. The average weight percentage of fluoride for all F-varnishes containing NaF ranged from 2.03 to 2.24% F, which is within 90% of the declared label concentration of 2.26% F. Analysis of the difluorosilane-containing product required an additional hydrolysis step. ANOVA found no significant difference between the 5% NaF varnishes at $p < 0.05$. This method for fluoride analysis yields reliable and reproducible results and can be used for a wide variety of F-varnishes. The standard uncertainty for this method is $\pm 4\%$. This method may become the basis for national and international standards that ensure the F-varnish products used in clinical practice have the fluoride content declared in the product literature.

Keywords

Fluoride; Fluoride measurement; Fluoride varnishes

Fluoride-releasing varnishes (F-varnish) are used to help prevent dental caries [ADA, 2006; Lawrence et al., 2008; Weintraub et al., 2006] and to treat dentin hypersensitivity [Beltran-Aguilar et al., 2000; CDC, 2001]. The number of F-varnish products in the US marketplace has increased from 4 in 2003 [Hazelrigg et al., 2003] to more than 35 in 2013 [Dental Product Shopper, 2013]. Fluoride-releasing dental varnishes typically contain 5% sodium fluoride (NaF, 2.26% F, 22,600 $\mu\text{g F/g}$) or 1% difluorosilane (0.1% F, 1,000 $\mu\text{g F/g}$) as their active ingredient suspended in a variety of resin/rosin mixtures that yields a range of handling properties. Other forms of fluoride such as sodium monofluorophosphate (MFP), amine fluoride ($\text{NH}_3\text{-F}$), potassium fluoride (KF), calcium fluoride (CaF_2), stannous fluoride (SnF_2) or a combination of these compounds could be envisioned within the varnish product. There are also a growing number of F-varnish products that contain adjunct ingredients such

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as xylitol, flavorings, and a variety of calcium-phosphate (CaPO_4) salts that are included to enhance fluoride release and up-take into enamel. These CaPO_4 additives have the possibility to interfere with the separation of fluoride for the analysis of the product. National and international standards organizations (ADA/ANSI and ISO) on dental products are preparing new standards that will include requirements on fluoride content for the total fluoride in the product. There is a need for the development of a simple, sensitive, and rapid analytical method that can be utilized as the standard method of analysis for total fluoride in F-releasing dental varnishes. This method may become the basis for national and international standards that ensure the F-varnish products used in clinical practice have the fluoride content declared in the product literature.

Shen and Autio-Gold [2002] described the measurement of total fluoride in three fluoride varnishes and reported a wide range of concentrations. This wide range was either due to a lack of product uniformity or inherent standard uncertainties related to the analytical methods used, or a combination of both. Since that time a large number of new F-varnish products have been introduced to the marketplace that are designed to release fluoride faster, reside on the tooth longer, or to have mobility around the oral cavity. Because F-varnishes have such a wide variety of compositions the development of a single method that can accurately determine the total fluoride content in commercially available F-varnishes is difficult. This is why we feel it is important to provide methods that can be used to assess the fluoride content for this wide variety of products.

We developed and tested a simplified sample preparation that can be used to analyze the fluoride content of complex samples. The basic approach is to dissolve the nonaqueous portion of the sample (resins and rosins) which does not contain active fluoride into chloroform, a hydrophobic organic phase. The sample-chloroform mixture is then mixed with an aqueous buffer to dissolve the fluoride complexes into an aqueous phase. The buffer serves several functions including chelating cations to release fluoride ions from ion pairs, and adjusting the ionic strength and pH to create the optimum conditions for fluoride ion-selective electrode analysis. The buffer we have found that meets our needs is marketed as Total Ionic Strength Adjusting Buffer II (TISAB[®]-II, Sigma-Aldrich, St. Louis, Mo., USA). TISAB-II contains high concentrations of cation chelators which facilitate hydrolysis of fluoride-ion pairs by releasing fluoride ions from any cation-fluoride complexes that may be in the product. An equivalent buffer solution can be made following the recipe described in ASTM International [2010]. This sample preparation sets our method apart from other methods in the literature. Our goal was to develop a reliable method to measure total fluoride concentration [F] in a wide variety of NaF and fluorosilane-based F-varnishes. We analyzed six types of F-varnishes which, according to package labels, contain 5% sodium fluoride (2.26% fluoride) with the exception of the fluorosilane product, which contains 1% difluorosilane (0.1% fluoride).

Methods

Sample Preparation

The method was evaluated by measurement of total fluoride in six types of commercially available fluoride-releasing dental varnishes that contained difluorosilane or sodium fluoride

either alone or in combination with calcium phosphate salts. Three of the products analyzed contained CaPO_4 salts as tricalcium phosphate, amorphous calcium phosphate, or a mixture of calcium sulfate and potassium phosphate. Other potentially confounding ingredients included protein, wax, xylitol, flavorings, colorings, thickeners and solvents such as ethanol, ethyl acetate, hexane, and glycerin.

The six varnish types employed in this investigation included two products that contained sodium fluoride alone (NaF-A and NaF-B), that had very different carrier matrices. Three contained sodium fluoride in combination with calcium phosphate: NaF with amorphous calcium phosphate forming salts (NaF/ACP), NaF with tricalcium phosphate (NaF/TCP), and NaF with casein phosphopeptide-amorphous calcium phosphate complex (NaF/CCP-ACP). The sixth varnish contained 0.1% difluorosilane ($\text{F}_2\text{H}_2\text{Si}$) as its fluoride source. Samples were taken from the individual varnish types within a single lot or reference number and within the label expiration assigned by the manufacturer.

To achieve product homogeneity, we mixed the varnish in the packaging prior to dispensing aliquots of varnish product. Samples were mixed a second time using either a stainless-steel spatula or the applicator brush provided before dispensing the varnish product to the digestion vial. For the product packaged in squeeze tubes, after kneading the tube for a few minutes to mix the product as best as possible, approximately 1 inch of product was dispensed and discarded from every new tube to ensure product homogeneity; samples were obtained from the remaining varnish product.

Sample Digestion

After testing, we determined that the required ingredients for sample preparation should be added in the following order: First, on a freshly tared balance, we added 0.05–0.15 g (1–3 drops) of the varnish product to a chloroform-resistant scintillation vial with 25 ml or greater capacity. We found that adding the sample directly to the vial allows for a decreased loss via evaporation and resulted in an easier and more accurate record of varnish mass. The $\text{F}_2\text{H}_2\text{Si}$ varnish samples required this immediate attention after dispensing when determining the F-varnish mass because the solvent of this product evaporates quickly. We placed approximately 4 ml (± 0.5 ml) of chloroform to each sample vial and swirled the contents to dissolve the varnish. After the varnish product was dissolved completely, we added 10.0 ml (± 0.1 ml) TISAB to dissolve and hydrolyze the fluoride complexes in the products. We observed that the addition of TISAB polymerizes the resin in some of the varnish products resulting in the formation of small beads. We determined that at this point in the dissolution phase, all contents should be vigorously shaken by hand until all of these hydrophobic beads of resin are disrupted. Following this mixing, the vials were stirred at 550 rpm overnight at room temperature. The following day, the stir plate was turned off and the samples were left to sit for at least 2 h to allow the chloroform and TISAB to separate. The top aqueous solution layer was then ready for fluoride measurement.

We found that after the separation phase of the NaF/ACP and NaF-A samples exhibited three layers, the chloroform layer, a white colored layer in the aqueous phase, and a clear aqueous phase. Prior to measurement, the two aqueous layers were lightly mixed ensuring supernatant homogeneity. After testing we determined that if the cloudy layer forms within

the chloroform layer, as occurs with the NaF/CCP-ACP samples, only the supernatant layer should be analyzed.

For one product we found that the rosin did not fully dissolve in the chloroform, leaving small clumps of residue at the interface of the chloroform and aqueous layers. The addition of an additional 4.0 ml of chloroform and an additional mixing step of vigorously shaking prior to stirring overnight did not dissolve the clumps. Follow-up analysis of these clumps found that they did not contain significant amounts of fluoride.

For the F₂H₂Si samples we found that the addition of 1.0 ml 1 mol/l potassium hydroxide (KOH) solution was required as an initial preparatory step to hydrolyze the fluorosilane prior to the addition of 10 ml TISAB for the dissolution of fluoride ion.

Sample Analysis

A fluoride ion-selective electrode was calibrated at room temperature following the manufacturer's instructions over the range of 1×10^{-5} to 1×10^{-2} mol/l NaF. The calibration plot, log[F mol/l] versus potential (mV), was linear and had a slope that fell within the range of -57 to -59 mV per decade [F⁻] mol/l at room temperature.

For the sample analysis, a blank solution containing 10.0 ml deionized water plus 9.0 ml TISAB solution was prepared for each sample to be analyzed. The blank solution to be used for the F₂H₂Si samples was made with 9.9 ml water plus 9.1 ml TISAB solution to maintain the same dilution as the other sample types. The aqueous phase of all samples was gently mixed prior to sample analysis to ensure supernatant homogeneity; 1.0 ml of the mixed varnish sample supernatant was added to the blank solution and analyzed via the calibrated standard fluoride ion-selective electrode. The fluoride concentration of the diluted sample was calculated from the standard curve and that value multiplied by the dilution factor of 100 and divided by the mass (g) of the varnish in the sample to calculate the fluoride concentration of the product in moles F/gram product. The fluoride concentration (mol/g) multiplied by 19,000 yields the fluoride concentration in micrograms F per gram of product (ppm F); this quantity can be multiplied by 2.21 to calculate the concentration of sodium fluoride in micrograms NaF per gram of product (ppm NaF).

A paired two-sample for means t test was used to determine if there were significant differences between the measured total fluoride concentration and the concentration value listed on the label. ANOVA was used to determine if there were significant differences in the mean total fluoride concentration between comparable 5% NaF-containing products.

Results

The mean percent of total fluoride within commercially available F-varnishes is given in table 1. All products contained at least 90% of the label amount of fluoride concentration. During the separation phase, a third layer of resin residues forms within the chloroform layer of many F-varnishes. For example, as a result of the varnish chemistry that allows for a protective coating of the calcium within the varnish, a thick white gelatinous layer within the

chloroform layer was observed in the NaF/TCP varnish. Figure 1 shows the separated layers for the products.

Paired two-sample for means t test found that the total fluoride concentration values for three varnishes (NaF-A, NaF-B, and F₂H₂Si) were significantly lower than the stated label values ($p < 0.05$). The significant differences for the NaF-A and NaF-B products are a result of small variations in the repeated sample measures. The current draft of international standard ISO/DIS17730 'Dentistry – Fluoride Varnishes' requires that the total fluoride content in F-varnishes do not deviate by more than 20% from the stated amount on the package. Even though the F-varnishes had less fluoride than stated on the labels the measured values for the NaF-A and NaF-B products were within 10% of the label value and thus well within the acceptable range. The F₂H₂Si F-varnish was not treated with KOH prior to fluoride extraction; the fluoride concentration of the samples that were treated with KOH was not significantly different from the product label concentration ($p > 0.05$). ANOVA found no significant difference in the fluoride concentrations between the 5% NaF varnishes ($p < 0.05$).

Discussion

The method is shown to be acceptable for the analysis of a wide variety of F-varnishes. The use of multifunctional TISAB to chelate fluoride-binding cations, as well as providing ionic strength and pH buffering to extract the fluoride from the products simplifies the analysis. TISAB contains high concentrations of 1,2-cyclohexyl-enediaminetetraacetic acid and acetic acid that are cation chelators which would otherwise form cation-fluoride complexes. The chelation of the cations releases fluoride ions from any fluoride ion pairs that may be in the product. Additionally, the TISAB provides the ionic strength and pH buffering needed for the ion-selective electrode analysis of total fluoride concentrations. The use of TISAB for the sample digestion and for fluoride ion-selective electrode analysis simplifies the method and reduces methodological errors.

In the development of this method we investigated several options for the protocol. When determining the order of adding ingredients for the sample digestion we thought that we could combine the chloroform and TISAB together in a vial to stir the mixture rapidly to create a vortex, and then add the varnish product into the vortex. We hoped to avoid the polymerization reaction that occurs when some F-varnish products contact water. Unfortunately, this approach made it difficult to determine the exact mass of F-varnish dispensed into the vortex, and it did not completely avoid the polymerization upon contact with the aqueous phase. Others have evaluated adding the F-varnish product directly to a mixture of TISAB and chloroform or to the chloroform only and they report that adding the product to the chloroform before adding TISAB yielded better results than adding the F-product to the TISAB-chloroform mixture [Flanigan et al., 2013]. We found that the determination of the mass of F-varnish added to the chloroform was difficult because the chloroform had significant evaporation during the time we weighed the sample. This introduced a large error in the analysis, which leads to an overestimate of the fluoride concentration.

We found that stirring the sample for an extended period of time (overnight or at least 8 h) greatly reduced variability. It is probable that any fluoride binding to cations or entrapment within the nonaqueous polymers takes a longer time to free the fluoride. We allow the samples to separate for several hours before taking aliquots of the aqueous layer for analysis. As seen in figure 1 the separation of the samples often yields three layers. We analyzed the compositions of the three layers and found that for the NaF/ACP product the white layer between the chloroform and TISAB contained fluoride presumably as calcium-fluoride complexes which had settled. This layer was easily mixed into the TISAB layer with gentle mixing of the sample, the fluoride concentrations measured for these samples were not significantly different from the concentration declared on the product label. The white layer that appeared to be between the chloroform and TISAB for the NaF/CCP-ACP product was actually in the chloroform and seems to be proteinaceous in nature. This layer did not contain fluoride. Finally, we did not find any significant amounts of fluoride in the chloroform layer even if there appeared to be insoluble F-varnish components in that layer. This possibility was reported by Shen and Autio-Gold [2002] when they first dissolved the product (package and all) into chloroform and then mixed the solution with water.

An additional hydrolysis step was needed for the product that contains difluorosilane. We found that the addition of 1.0 ml of 1 mol/l KOH prior to stirring overnight in the preparatory stages of this method allowed the fluoride ions to become available for measurement via fluoride ion-selective electrode. It is anticipated that as new F-varnish products are introduced into the marketplace there will be ingredients such as difluorosilane that will require additional steps in the sample preparation phase of the analysis. This method is designed such that additional steps could be added to the protocol without greatly changing the overall analytical method of separating the nonaqueous portions of the product from the water-soluble portion and using the strong chelating buffer to hydrolyze any fluoride ion pairs for the analysis of the samples.

An analysis of errors from evaluation of each step of the procedure shows that there are several steps where measurement is critical. The first step of measuring the mass of the sample to be analyzed can introduce as much as 2% error with a variation of ± 0.002 g mass of F-varnish. The method indicates determination of the F-varnish product mass to ± 0.0001 g; however, some samples have a very volatile carrier making it difficult to measure the amount of F-varnish dispensed with high accuracy. The other potential source of error in the method is the measurement of fluoride concentration via fluoride ion-selective electrode. The fluoride ion-selective electrode analytical system will drift as much as 1 mV over the course of several hours. At the typical concentration of the diluted sample a ± 1 mV drift will cause a variation of 900 $\mu\text{g/g}$ for samples of 22,600 $\mu\text{g/g}$ fluoride concentration for approximately $\pm 4\%$ standard uncertainty. Each of these sources of error (or uncertainty) can be minimized through careful sample handling during the preparation for the sample digestion, careful sample pipetting and including measurement of fluoride standards interspersed between sample measurements. Due to these sources of error and fluoride ion-selective electrode variations over time it is reasonable to assume that the standard uncertainty of this method is $\pm 4\%$, which is equal to the standard deviation of the repeated measures for the samples. We suggest the inclusion of an internal standard at 5% NaF

(50,000 µg/g NaF), which is treated in exactly the same manner as the F-varnish samples as a form of quality control for the method.

This method for the determination of total fluoride concentration in F-varnishes yields reliable and reproducible results and can be used for a wide variety of F-varnishes. The estimate for standard uncertainty as derived from an evaluation of uncertainties and the average standard deviation of the analyses is $\pm 4\%$. This method may become the basis for national and international standards that ensures the F-varnish products used in clinical practice have the fluoride content declared in the product literature.

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Fig. 1. Separation layers of the varnish products. A = Aqueous layer; I_a = insoluble layer at the aqueous interface; I_c = insoluble layer at the chloroform interface; I_s = insoluble layer in the chloroform layer; C = chloroform layer.

Table 1

Fluoride concentration in varnish products

Varnish	[F], %		NaF, %		n ³
	average	SD	average	SD	
NaF-A	2.03	0.04	4.48	0.10	05
NaF/ACP	2.23	0.04	4.94	0.10	09
NaF/TCP ¹	2.20	0.29	4.87	0.64	09
F ₂ H ₂ Si ²	0.076	0.004	no NaF	no NaF	10
F ₂ H ₂ Si-KOH	0.100	0.002	no NaF	no NaF	06
NaF-B	2.03	0.07	4.48	0.16	08
NaF/CCP-ACP	2.24	0.03	4.95	0.07	09

¹The large standard deviation (SD) may reflect product inhomogeneity.²These F₂H₂Si samples were not hydrolyzed with base, the lower measured fluoride concentration reflects incomplete hydrolysis of the difluorosilane.³n values are different due to combining data for products measured on several occasions and measurement of products which were acquired later in the study.