

## TiF<sub>4</sub> Varnish—A <sup>19</sup>F-NMR Stability Study and Enamel Reactivity Evaluation

Carolina Bezerra Cavalcanti NÓBREGA,<sup>a</sup>  
Fred Yukio FUJIWARA,<sup>b</sup> Jaime Aparecido CURY,<sup>a</sup> and  
Pedro Luiz ROSALEN<sup>\*a</sup>

<sup>a</sup>Departamento de Ciências Fisiológicas, Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas; Caixa Postal 52, Piracicaba, CEP 13414-903, Brasil; and  
<sup>b</sup>Instituto de Química, Universidade Estadual de Campinas; Caixa Postal 6154, CEP 13084-862, Brasil.

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The aim of this study was to develop a titanium tetrafluoride (TiF<sub>4</sub>) varnish and evaluate the stability of the formulation and its reactivity with dental enamel. The varnish was prepared in a resinous matrix using ethanol 96% as solvent. Samples (*n*=45) were aged at 65 °C and 30% of relative humidity (RE n° 01/05—ANVISA) and after 3, 6, 9 and 12 months, nine samples were removed for evaluation and compared with fresh samples. Chemical stability of TiF<sub>4</sub> varnish was determined by <sup>19</sup>F-NMR and the reactivity of the formulation was quantified by formation of fluoride loosely (CaF<sub>2</sub>) and firmly bound (fluorapatite; FA) to enamel. For reactivity comparisons, a varnish without TiF<sub>4</sub> was used as control. The loss of soluble fluoride was about 0.9% after one year of storage. The values of the reactivity (mean±S.D.) of fresh, aged at 3, 6, 9 and 12 months and control samples were: CaF<sub>2</sub> (μg F/mm<sup>2</sup>): 89.3±27.5<sup>a</sup>; 54.5±14.3<sup>b</sup>; 51.2±29.8<sup>b</sup>; 69.3±21.3<sup>a</sup>; 48.0±27.4<sup>b</sup>; 0.10±0.07<sup>c</sup>, FA (μg F/g): 2477.5±1044.0<sup>a</sup>; 2484.8±992.0<sup>a</sup>; 2580.0±1383.9<sup>a</sup>; 2517.2±929.9<sup>a</sup>; 2121.0±1059.2<sup>a</sup>; 330.0±180.0<sup>b</sup>, respectively. Means followed by distinct letters were statistically different (*p*<0.05). After one year of storage, the formulation was chemically stable and the levels of FA were maintained. However there was an initial decrease in the ability to form CaF<sub>2</sub>.

**Key words** titanium tetrafluoride; drug stability; fluoride varnish

Although professionally applied topical fluoride treatment has been used for caries control for many years, there is an interest in finding new compounds for this purpose.<sup>1,2</sup> TiF<sub>4</sub> (titanium tetrafluoride) is a fluoridated compound which has low values of pH (*ca.* 1–2)<sup>3</sup> in water or alcohol solutions. In addition to the pH values, titanium corrosion resistance and the capacity to form an oxide coating on enamel could be responsible for the results related to caries process.<sup>1,2,4–7</sup> However, in spite of the promising results, this compound has not yet been used in clinical procedures due to the lack of stability in water solutions.<sup>8</sup>

TiF<sub>4</sub> hydrolysis processes in water have been described, with emphasis on the formation of many fluoride complexes.<sup>4,6</sup> In addition to the stability and physical chemical properties of fluoridated agents, the efficacy of these products depends on the capacity to react with enamel forming reaction products, which can be loosely (calcium fluoride) or firmly (fluorapatite) bound to enamel and could interfere with the de- and re-mineralizing phases of the caries process.<sup>7</sup> The aim of this study was to produce a TiF<sub>4</sub> varnish and evaluate the chemical stability and enamel reactivity during one year period.

### Experimental

**Varnish Preparation and Aging** The TiF<sub>4</sub> varnish was prepared by dissolving TiF<sub>4</sub> (*ca.* 4%) and resin products in ethanol 96%. A homogeneous solution was obtained by slowly adding the solids to ethanol with vigorously stirring. Next, the viscous solution was placed in plastic containers (*n*=45), protected from light exposure and maintained in an aging process at 65 °C and 30% RH (relative humidity).<sup>9</sup> After 3, 6, 9 and 12 months, nine samples were removed for analyses and compared with fresh samples.

**NMR Spectra** The fresh and aged TiF<sub>4</sub> varnish formulations were analyzed by <sup>19</sup>F-NMR using a Bruker AC300 (282.4 MHz) and an Advance DPX (235.4 MHz) spectrometer in 5 mm co-axial tubes at –30 °C. CFCl<sub>3</sub> was placed in the center coaxial tube as a chemical shift reference ( $\delta$ =0 ppm) and as a reference for quantitative analyses.

**Reactivity with Enamel** The reactivity of the TiF<sub>4</sub> varnish formulation was evaluated using 15 bovine enamel blocks (3×3×2 mm) per group/time. A varnish without TiF<sub>4</sub> was used as a negative control. All the block surfaces were protected with dental wax except for the enamel surface. The treatments were applied to the enamel for 4 min, the excess of varnish on the block was removed with acetone and the enamel surface was washed with distilled water. The blocks were placed in individual tubes containing 0.5 ml 1.0 M KOH to extract the loosely bound fluoride formed on enamel (CaF<sub>2</sub>).<sup>10</sup> After 24 h under agitation at room temperature, 0.5 ml of TISAB II, pH 5.0, containing 1.0 M HCl was added to the tubes. The blocks were washed with deionized water and transferred to another tube to extract, by acid etching, the firmly bound fluoride (FA) formed. Then 0.5 ml of 0.5 M HCl was added to the tubes and after 60 s under agitation, the acid was buffered with 0.5 ml of TISAB II (Total Ionic Strength Adjustment Buffer), pH 5.0, added 20 g NaOH/l.

Fluoride as CaF<sub>2</sub> and FA (fluorapatite) were determined with an ion-specific electrode (Orion 96-09) and an ion analyzer (Orion 720 A), which were previously calibrated.

**Statistical Analysis** The data of fluoride formed were analyzed by ANOVA and Tukey test, and the significance limit was set at 5%.

### Results

A typical <sup>19</sup>F-NMR spectrum is shown in Fig. 1 and the complexes identified are shown in Table 1. Low temperatures are used to obtain the spectra because the spectra obtained at ambient temperature presented very broad peaks due to chemical exchange of fluorines between the complexes. Peaks from fluorines in the same complex were identified by their coupling constants or by an F–F COSY experiment and the chemical shifts were calibrated using CFCl<sub>3</sub> as the external standard. The areas under the individual peaks were obtained by a deconvolution procedure where a Lorentzian line-shape is adjusted to each peak. The Bruker Topspin 1.3 software was used to process the spectra. The relative intensities of the fluorines in the Ti complexes determined are shown in Fig. 2. The percentage of fluoride loss after 1 year was approximately 0.9%. It is calculated by comparing the total intensity of the peaks of all complexes with the peak of the CFCl<sub>3</sub> reference in the coaxial tube.

The amount of CaF<sub>2</sub> formed on the enamel initially decreased at the beginning of storage and then remained relatively constant. However, the varnish keeps its capacity to form FA in enamel during all experimental time. These results are presented in Table 2.

### Discussion

Toothpastes, varnishes, mouth rinses and gels are popular local delivery systems used in dentistry<sup>11–14</sup> and there are several commercially available products for professional topical application. Teeth varnish is a delivery system, which is widely used today to minimize the risks of fluoride over-ingestion. The high capacity of the varnish to keep the active

\* To whom correspondence should be addressed. e-mail: rosalen@fop.unicamp.br

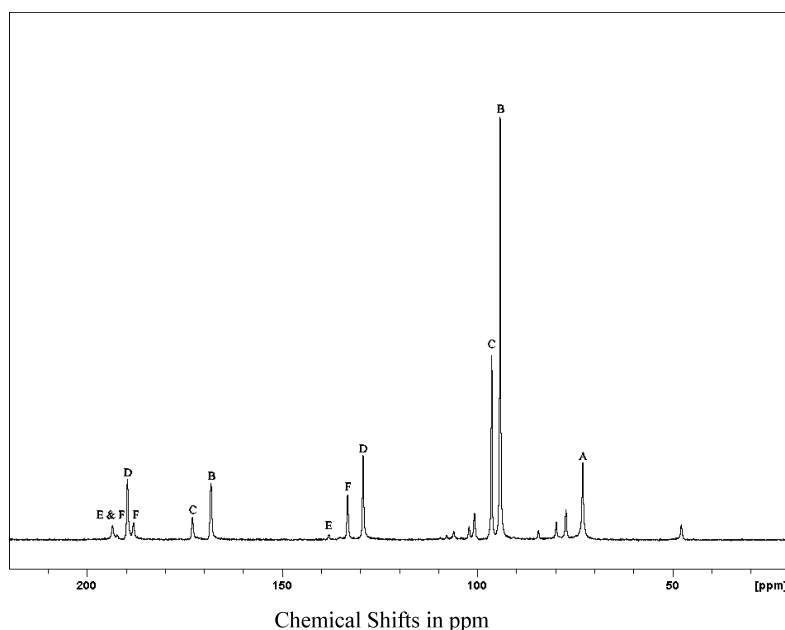


Fig. 1.  $^{19}\text{F}$ -NMR Spectra of the Freshly Prepared  $\text{TiF}_4$  Varnish at  $-30^\circ\text{C}$

Table 1. Chemical Shift Assignments of Ti Complexes in the Varnish<sup>3,4,6,19,20</sup>

Peaks identification	$\text{TiF}_4$ complexes	Chemical shifts in ppm (External reference $\text{CFCl}_3$ )
A	$[\text{TiF}_6]^{2-}$	73.4 (6F)
B	$[\text{TiF}_5 \cdot \text{H}_2\text{O}]^-$	168.8 (2F); 94.6 (3F)
C	$[\text{TiF}_5 \cdot \text{EtOH}]^-$	173.5 (2F); 97.0 (3F)
D	$\text{TiF}_4 \cdot 2\text{H}_2\text{O}$	190.1 (2F); 129.9 (2F)
E	$\text{TiF}_4 \cdot 2\text{EtOH}$	194.0 (2F); 138.6 (2F)
F	$\text{TiF}_4 \cdot \text{H}_2\text{O}, \text{EtOH}$	194.0 (1F); 188.6 (1F); 133.7 (2F)
—	Not identified	108.5; 106.5; 102.6; 101.4; 84.9; 80.2; 77.8; 48.2

Table 2. Enamel Fluoride Formed (Mean  $\pm$  S.D.,  $n=15$ ) as  $\text{CaF}_2$  ( $\mu\text{g F}/\text{mm}^2$ ) and FA ( $\mu\text{g F}/\text{g}$ )

Period	$\text{CaF}_2$ ( $\mu\text{g F}/\text{mm}^2$ )	FA ( $\mu\text{g F}/\text{g}$ )
First day	$89 \pm 28^a$	$2500 \pm 1000^a$
3 months	$55 \pm 14^b$	$2500 \pm 1000^a$
6 months	$51 \pm 30^b$	$2600 \pm 1400^a$
9 months	$69 \pm 21^a$	$2500 \pm 930^a$
12 months	$48 \pm 27^b$	$2100 \pm 1100^a$
Negative control	$0.10 \pm 0.07^c$	$330 \pm 180^b$

Significant differences among periods, for each reaction product, are indicated by different letters ( $p < 0.05$ ). Data analyzed by ANOVA and Tukey test.

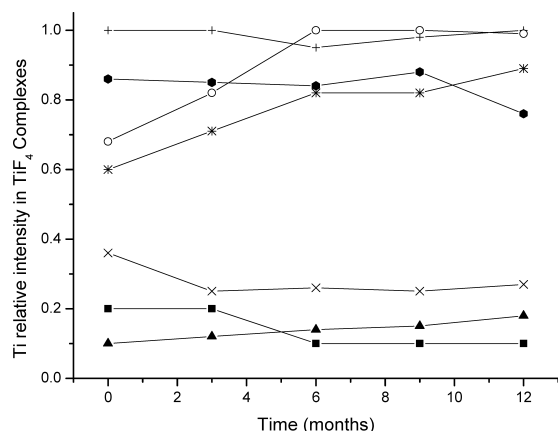


Fig. 2. Variations in the Relative Intensities in the Ti Complexes during the Storage Period

+,  $\text{TiF}_4 \cdot \text{H}_2\text{O}, \text{EtOH}$ ; ●,  $[\text{TiF}_5 \cdot \text{EtOH}]^-$ ; ○,  $[\text{TiF}_5 \cdot \text{H}_2\text{O}]^-$ ; \*,  $\text{TiF}_4 \cdot 2\text{H}_2\text{O}$ ; x,  $\text{TiF}_4 \cdot 2\text{EtOH}$ ; ■, total intensity of unidentified peaks; ▲,  $[\text{TiF}_6]^{2-}$ .

compound in contact with teeth enamel, the secure use of high concentrations of fluoride compounds and the advantage of using ethanol as solvent were the main properties considered to produce this varnish. In relation to stability, the

maximum percentage loss suggests by Brazilian Sanitary Authorities<sup>9)</sup> is 5%. The  $\text{TiF}_4$  varnish after aging for one year presented a 0.9% loss of soluble fluoride compounds when compared to a fresh varnish and therefore can be considered as a stable formulation.

Solutions of  $\text{TiF}_4$  in ethanol were reported to contain primarily the  $\text{TiF}_4 \cdot 2\text{EtOH}$  complex.<sup>15,16)</sup> Species as  $[\text{TiF}_5 \cdot \text{EtOH}]^-$ ,  $[\text{TiF}_5 \cdot \text{H}_2\text{O}]^-$ ,  $\text{TiF}_4 \cdot 2\text{H}_2\text{O}$  and  $\text{TiF}_4 \cdot \text{H}_2\text{O}, \text{EtOH}$  seems to be the major products in the reaction and the percentage of fluorine complexes with water ligands has the tendency to increase during time (Fig. 2). The percentage of unknown species were small (Fig. 2) and could be oxides such as  $[\text{TiOF}_4]^{2-}$ ,  $[\text{TiOF}]^+$ ,  $\text{TiOF}_2$  and  $[\text{TiOF}_3]^-$ , that are present in hydroxide fluoride solutions.<sup>4)</sup> Buslaev Y. A., *et al.*<sup>4)</sup> studied the hydrolysis of  $\text{TiF}_4$  in ethanol with various amounts of water and observed that several hydrolysis products were formed; most of them are of low concentration and only  $[\text{TiF}_5 \cdot \text{H}_2\text{O}]^-$  and  $[\text{TiF}_5 \cdot \text{EtOH}]^-$  were positively identified. In all of the solutions,  $\text{TiF}_4 \cdot 2\text{EtOH}$  was found, which is the same observed in this study. Spectra of  $\text{TiF}_4$  in absolute ethanol were used to confirm the assignment of the complexes, which do not have water as a ligand. The peaks due to the unidentified compounds were not observed in solutions prepared with absolute ethanol, which indicates that these

compounds are a result of reactions of  $\text{TiF}_4$  with water.

In relation to reaction products shown in Table 2, the maintenance of FA levels is important to reduce mineral loss during caries process. However, the decrease in  $\text{CaF}_2$  formation could interfere in inhibition of the dynamic of caries process, due to its capacity to act as a pH-controlled reservoir of ions on enamel and dental plaque that can be released during cariogenic challenges.<sup>17,18)</sup>

### Conclusion

In conclusion, the varnish can keep fluoride compounds stable for a year. Furthermore, the varnish was able to maintain the levels of enamel uptake as FA although it was not capable of keeping the levels of calcium fluoride. Further studies are required to explain how this decrease in one of enamel fluoride reservoirs could interfere in factors related to caries process.

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