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Characterization of a new TiF_4 and β-cyclodextrin inclusion complex and its *in vitro* evaluation on inhibiting enamel demineralization

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ABSTRACT

Titanium tetrafluoride (TiF_4) is an effective but instable caries preventive agent. As the stability problems could be minimized through the use of drug carriers this study aimed to prepare and characterize a new TiF_4 nanoinclusion complex and to evaluate its potential in inhibiting enamel demineralization under pH cycling conditions. The TiF_4 nanosystems were prepared using β-cyclodextrin (βCD) and sodium montmorillonite (MMTNa). Bovine enamel blocks ($n = 48$) with known surface microhardness (SMH), were randomly assigned to 4 groups ($n = 12$) and submitted to one of the following treatments: distilled deionized water (as negative control) and solutions containing 1% βCD, 1% TiF_4 and TiF_4:βCD. The solutions were blinded applied once on the blocks with a microbrush® on the surface for 1 min before pH-cycling. After that, samples were reavaluated by SMH, %SMH loss, cross-sectional microhardness (CSMH), scanning electron microscope (SEM) and energy dispersive spectrometry (EDX). The inclusion complex of TiF_4:βCD offered better protection against demineralization in the subsurface. The SEM analysis showed that TiF_4 and TiF_4:βCD samples presented the most intact enamel than the control. The EDX analysis identified titanium in TiF_4 and TiF_4:βCD groups. TiF_4:βCD has higher potential on inhibiting demineralization in the inner enamel. TiF_4:βCD is a new alternative to TiF_4 stabilization in order to reduce enamel subsurface demineralization.

1. Introduction

Professionally applied topical fluoride treatment has been used for caries control for many years. However, there is an interest in finding new compounds for this purpose. 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 can interfere with the de- and remineralizing phases of the caries process.[1]

In vitro studies have shown that a single treatment of titanium tetrafluoride (TiF_4) solution[2–4] or varnish[3] on sound enamel is effective in preventing the formation of artificial

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carious lesions. TiF_4 solution is a fluoridated compound which has a low pH value,[5,6] and forms numerous fluoride complexes during the process of hydrolysis.[7–10] Despite its positive effects, solutions of TiF_4 alone are not sufficiently stable for treatments.

However, stability problems can be minimized by using suitable drug carriers, such as supramolecular aggregates, nanocarriers and cyclodextrins (CDs). In the pharmaceutical field, these substances can bind to drugs, modifying their chemical stability as well as other properties such as solubility, dissolution rate, and bioavailability. It has scientific evidence on the complexation with different cyclodextrins have reported clear proof of these advantages.[11] At the same time, good results can be obtained using nanocarriers with antibacterial activity, such as sodium montmorillonite (MMTNa), the main constituent of bentonite (60%). A good inclusion complex is possible with this nanocarrier and several different metal substances, such as TiF_4.[12] Thus, the aim of this study was to prepare and characterize a new TiF_4 nanoinclusion complex and to evaluate its potential in inhibiting enamel demineralization under pH cycling conditions.

2. Materials and methods

2.1. Materials

Ethanol 95% (Merck, Darmstadt, Germany), sodium montmorillonite (Acros Chemical, Belgium, Wisconsin, USA), β-cyclodextrin (Wacker GmbH, München, Germany), and titanium tetrafluoride (Sigma, St. Louis, Minnesota, USA), were all of pharmaceutical grade. Solutions were prepared with purified water obtained using a Milli-Q system (Millipore, Billerica, Massachusetts, USA).

2.2. Preparation of cyclodextrin complexes

The inclusion complexes of TiF_4:cyclodextrin (TiF_4:CD) were prepared by kneading, solubilization and freeze-drying at molar ratios of 1:1, 1:2, 1:3 and 1:4. Physical mixtures were prepared by mixing CD and TiF_4 in a mortar at the same molar ratios. β-Cyclodextrin (βCD), methyl-β-cyclodextrin (MβCD) and hydroxy-propil-β-cyclodextrin (HPβCD) were used in this study. Using the kneading method, CD and TiF_4 were mixed in a mortar for 5 min. An ethanol:water (70:30; v/v) solution was added and the system was mixed for 30 min to obtain a homogeneous paste. The paste was dried under reduced pressure and the granulometry adjusted using a 40 mesh sieve. Following the solution method, the appropriate proportions of TiF_4 and CD were mixed in 20 mL of distilled water with a magnetic stirrer for 72 h. The samples were frozen in liquid nitrogen and lyophilized. The particle size was also calibrated with a 40 mesh sieve. The inclusion yield was calculated by UV spectroscopy.

2.3. Preparation of clay based nanosystems and complexes

The prepared complex stability was obtained through fluoride dosage and pH of TiF_4 and inclusion complex 500 ppm fluoride solutions. Samples were stored under 40 ± 2 °C/75 ± 5% RH for 30, 60 and 90 days. The fluoride content and pH parameters were obtained in Analyser pH/ion 450 M (mV) fluoride electrode (ORION 9609BNWP).

TiF_4:MMTNa nanosystems were prepared following the solution method with different cation exchange capacity (CEC) values, 100%, 80% and 60% of the total MMTNa. The CEC value used was 100 mequiv. of cation to 100 g of MMTNa.[13] The inclusion reactions were performed in triplicate for different periods (1, 18, 24 and 48 h), stirring at room temperature. The reaction mixtures were centrifuged at 4000 rpm for 40 min and the precipitates dried in a vacuum desiccator. The inclusion yield was calculated by UV spectroscopy.

2.4. Characterization of nanosystems

These nanosystems were characterized by X-ray powder diffraction (XRPD), Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). XRD patterns of nanosystems, physical mixtures and pure substances were recorded with a Rigaku Miniflex diffractometer BD11197 (Rigaku, Shibuya-Ku, Tokyo, Japan) using CuK radiation with a current of 30 mA, voltage of 40 kV and a 2θ angle between 2° and 20°. FTIR spectra were collected by an IR Prestige-21 Shimadzu A210045 (Shimadzu, Nakagyo-ku, Kyoto, Japan) spectrometer using 2% KBr pellets and a wave number between 4000 and 400 cm^{-1}. DSC analyses were carried out with a DSC 882e Mettler-Toledo machine using hermetically sealed aluminium pans under a nitrogen flow of 28 mL min^{-1} and heating rate of 10 °C min^{-1}.

2.5. Studies for inhibiting enamel demineralization

2.5.1. Experimental design

This blind and randomized study evaluated *in vitro*, using a previously validated protocol,[14] the inhibiting enamel demineralization capability of a new inclusion complex (TiF_4:β-Cyclodextrin). Groups with 12 sound bovine enamel blocks each were randomly chosen to evaluate the following 4 treatment groups: control (distilled deionized water [DDW]), a solution containing 1% β-cyclodextrin (βCD), a solution containing 1% TiF_4, and the experimental formulation containing TiF_4:βCD. The response variables studied were: enamel surface microhardness loss (%SML), cross-sectional microhardness (CSMH) and energy dispersive X-ray spectrometry (EDX) analysis. The blocks were also analyzed qualitatively by scanning electron microscope (SEM) after the pH-cycling regimens and treatments with the formulations.

2.5.2. Preparation of enamel blocks

Enamel blocks (4 mm × 4 mm × 3 mm) were obtained from 80 sound bovine incisors that had been stored in 2% formaldehyde solution.[15] The blocks were included in acrylic devices and polished with 600 and 1200 grade silicon carbide paper, followed by 3 and 1 μm diamond abrasive slurry (Buehler Ltd., Lake Bluff, Illinois, USA). The slabs were polished flat and 48 slabs were selected based on the baseline surface microhardness (SMH) (mean 321.35 ± 32.13 kg/mm^2). SMH was measured using a microhardness tester (HVS-1000, Time Group Inc., Beijing, China) with a Knoop diamond under a 50 g load for 5 s,

by making 5 indentations spaced 100 μm[16] from each other at the centre of enamel surface.

2.5.3. Treatment and demineralizing pH-cycling

The forty-eight blocks were randomly assigned to 4 groups ($n = 12$) and submitted to one of the following treatments: distilled deionized water (as negative control) and solutions containing 1% βCD, 1% TiF_4 and TiF_4:βCD. The solutions were blinded applied only once on the surface of the blocks with a microbrush® and left for 1 min. After this, the blocks were rinsed with DDW, and dried with soft paper. Then the pH-cycling started.

The pH-cycling regimen took 8 days, and the blocks were kept at 37 °C for 4 h in the demineralizing solution and 20 h in the remineralizing solution, according to Queiroz et al.[17] The demineralization solution used contained 0.05 M acetate buffer pH 5.0, 1.28 mM Ca, 0.74 mM P, 0.03 μg F/mL. The remineralizing solution contained 1.5 mmol/L Ca, 0.9 mmol/L P, 150 mmol/L KCl, 0.05 μg F/mL in 0.1 mol/L Tris buffer, pH 7.0. The proportion of demineralizing and remineralizing solutions per area of block was 6.25 mL/mm^2 and 3.12 mL/mm^2, respectively. On the 4th day, the de- and re-mineralizing solutions were replaced by fresh ones. After the 8th day of the cycle, the blocks remained in the remineralizing solution for an additional 24 h until analysis.[17]

2.5.4. Microhardness analysis

After the treatments and demineralizing pH-cycling, enamel SMH was measured again by making 5 indentations spaced 100 μm from the baseline ones. The percentage of surface microhardness loss (%SML) was calculated using the following equation:

$$\%\text{SML} = \frac{\text{sound SMH} - \text{SMH after pH-cycling}}{\text{sound SMH}} \times 100.$$

2.5.5. Cross-sectional microhardness analysis

Subsequently, all enamel blocks were tested for cross-sectional microhardness (CSMH). They were longitudinally sectioned in the middle of the fragment with a cutting machine (Isomet, Buehler, Lake Bluff, IL, USA), resulting in two halves. One half was included in the stub and the cut surfaces were exposed and polished with 600 and 1200 grade silicon carbide paper. The CSMH analyses were performed by a microhardness tester (HVS-1000, Time Group Inc., Beijing, China) with a Knoop diamond and a 25 g static load that was applied for 10 s.[18]

Two sequences of 15 indentations were made at from 10 to 100 μm depth indentations at 10 μm intervals and from 100 to 200 μm depth at 20 μm intervals were made from the outer enamel surface, 100 μm apart. The treatments were compared for each depth.

2.5.6. Scanning electron microscopy/Energy dispersive X-ray spectrometry (SEM/EDX) analysis

These blocks were mounted on aluminium stubs and analyzed using scanning electron microscopy (JEOL-JSM; 6460LV, Tokyo, Japan). The topography of sectioned surfaces and the enamel surfaces were analyzed in backscattered electrons (BSE) at 20 kV voltage, low vacuum mode (45 Pa) to obtain images with a 1000× magnification.

The chemical analysis for the assessment of mineral content of the enamel on the surface and in the cross-section was performed using EDX with link and automatic image analyzer system Kontron. This analysis was performed to identify the chemical elements in the inner enamel after the experimental protocol. The elements analyzed were: Titanium, Oxygen, Calcium, Carbon, Sodium, Magnesium, Aluminium, Phosphorus, Chlorine, Silicon and Potassium.

2.6. Statistical analysis

The assumptions of equality of variance and normal distribution of error were checked with the Shapiro–Wilk test for all response variables. Student's paired *t*-test was used to compare the Knoop surface microhardness before and after pH-cycling of all treatments with a p value of 0.05.

For analyses of % SML the differences among treatments were analyzed by analysis of variance ANOVA 1-way followed by Tukey's *post hoc* test. For analyses of CSMH it was calculated the area under curve (ΔZ), the differences among treatments were analyzed by analysis of variance (ANOVA 1-Way) and Tukey's *post hoc* test. For all statistical analysis, the SPSS software, version 17.0 was used. Differences between means were considered significant when values of $p < 0.05$ were obtained.

3. Results

3.1. Preparation and characterization of TiF_4 and cyclodextrin inclusion complexes

The same molar ratio was used to obtain all cyclodextrin–TiF_4 inclusion complexes. After the oxidation test, only the βCD inclusion complex (TiF_4–βCD) did not show any oxidation when compared with the inclusions using methyl-β-cyclodextrin (MβCD) and hydroxy-propil-β-cyclodextrin (HPβCD). Moreover, only the 1:4 TiF_4–βCD molar ratio remained solid after lyophilization without sample darkening due to titanium oxidation. The material oxidation, due to the drying process observed during the preparation of the inclusion complexes by kneading, made this method unsuitable for TiF_4 complexation. Only the solubilization-freeze-drying method can be used for TiF_4 inclusion. The loss of crystallinity in the sample and displacement of the TiF_4 fusion endothermic peak in the differential scanning calorimetry (DSC) analysis were the major evidences of inclusion complex formation.

Stability results obtained for TiF_4 (500 ppmF) and TiF_4:βCD (500 ppmF) solutions stored under 40 ± 2 °C/75 ± 5% RH for 30, 60 and 90 days are presented in Table 1.

3.2. Preparation and characterization of clay based nanosystems

There was no significant difference in the interlamellar spacing value ($p = 0.0987$) among the TiF_4 nanosystems, regardless of the conditions for preparation. The titanium inclusion yields in the MMT-Na derivatives were around 35%, indicating material consumption. Experimental conditions with a shorter inclusion time and lower CEC (T1) were used to

Table 1 – Stability results obtained for TiF_4 (500 pmmF) and TiF_4:βCD (500 ppmF) solutions stored under 40 ± 2 °C/75 ± 5% RH for 30, 60 and 90 days.

	TiF_4 solution stability				TiF_4:βCD solution stability			
Period (days)	0	30	60	90	0	30	60	90
pH	2.47	2.04	1.72	1.21	4.95	4.87	4.70	4.66
Measure (mV)	−49	−33	−17	−14	−47	−45	−46	−42
Concentration (ppm)	512.86	341.04	175.12	149.88	490.94	470.04	480.61	438.11

check reproducibility and presented an average basal spacing of 15.11 ± 0.13 Å and an average yield of 35 ± 0.03%. The reaction of TiF_4 and MMT-Na was first described in this work and represents a potential alternative for controlling the dental demineralization–remineralization process. No differences were observed between the TiF_4 inclusion complex and the MMTNa interlamellar spacing indicating titanium absorption or its insertion in the MMTNa structure. MMTNa was used as a nanocarrier due its sustained release properties and synergistic antimicrobial activity. The nanosystems produced were characterized by X-ray powder diffraction (XRPD), DSC and Fourier transform infrared spectroscopy (FTIR). A decrease in the 2θ value observed in the XRPD analysis is an indicative factor of the inclusion process since a reduction in this angle is associated with an increase in basal spacing, which is related to drug inclusion. Therefore, it is possible to affirm that no intercalation product was obtained with the new titanium:MMTNa structure produced.

3.3. *Surface and cross-sectional microhardness*

Table 2 shows the analysis of enamel blocks with regard to SMH. The TiF_4 group presented smaller mineral loss when compared to all treatments ($p < 0.05$).

There was statistical difference between TiF_4:βCD and control group when the CMSH (ΔZ) was analyzed ($p < 0.05$), while the other groups were similar to control ($p > 0.05$). TiF_4:βCD was similar to TiF_4 and to βCD in reducing mineral loss ($p > 0.05$) (Table 2).

3.4. *SEM/EDX analysis*

The enamel samples from all groups were analyzed descriptively from the findings on SEM in order to observe the characteristics of the surface and subsurface enamel after treatment and pH-cycling.

The images of control and βCD groups (Fig. 2A and B) show that the enamel subsurfaces appear to be more porous than the images of the TiF_4 and TiF_4:βCD groups (Fig. 2C and D). The surface images revelled that only the control group had a large loss (Fig. 1A).

When comparing the subsurface images of the samples that received treatment with TiF_4 and TiF_4:βCD (Fig. 2C and D), the samples treated with TiF_4:βCD (Fig. 2D) looked more integrated. However, the images of the surface were very similar (Fig. 1C and D).

The chemical analysis (EDX) revealed the presence of the element titanium in all samples treated with TiF_4 and TiF_4:βCD on the surface (Fig. 1C and D), and subsurface (Fig. 2C and D). The presence of the element titanium was more evident on the surface than in the subsurface.

4. Discussion

The purpose of this study was to prepare, characterize and evaluate the effect of a new TiF_4:βCD inclusion complex solution to inhibit enamel demineralization using a pH-cycling model with bovine enamel.

The advantages of *in vitro* caries models are the facility and the agility in obtaining results. In addition better control can be exerted over the conditions for chemical remineralization within a given exposure period.[19] However, in some models, under-saturation could be driving the remineralization, leading to problems such as erosion. In order to avoid this problem, it is necessary to develop a pH-cycling model that simulates artificial enamel decay but does not produce an undersaturated solution.[20] Therefore, the pH cycling model was chosen for this study because it presents an F dose–response effect and it could be used to evaluate the anti-caries potential of various fluoride products used to inhibit enamel demineralization or to enhance enamel remineralization.[17]

The results obtained showed no obvious TiF_4 insertion in the MMTNa interlamellar space. According to Meng et al.[12] the MMTNa monolayer presents a size of approximately 0.96–0.97 nm. The size of the TiF_4 molecule is 0.55 nm whereas the lamella height is 0.96 nm, and the basal spacing of the nanosystem produced under optimum conditions is 1.51 nm. This value does not corroborate the value described for Ti^{4+} ion of 0.69 nm.[21] Juang et al.[21] evaluated the cationic exchange of MM-Na for Fe^{2+}, Cr^{3+} and Ti^{4+}, showed that the clay tetrahedral layer of Si^{4+} could possibly be exchanged for Ti^{4+} ion by doubling the CEC. Another hypothesis is that TiO_2 lodged in specific areas on the surface and in the interlayer spacing of the clay. However, Juang et al.[21] presented evidence that the Si^{4+}/Ti^{4+} exchange theory better explains the titanium consumption process. Moreover, Zhang et al.[22] described the fluorine inclusion with a basal spacing of 15 Å, similar to the results obtained with a 1 h inclusion time and 60% CEC (15.11 Å), confirming the Si^{4+}/Ti^{4+} exchange theory. The amount of titanium presented in the samples was 9.2% without fluorine detection measured by X-ray fluorescence. This result indicates that the 35% indirect inclusion yield obtained here is very realistic, considering that 37% of TiF_4 mass is Ti and the total amount of the Ti in this case is 13.26%, which is similar to that obtained by X-ray fluorescence.

Since TiF_4:MMTNa inclusion complex were not successful obtained, due to excessive oxidation of the titanium, a new Titanium-clay nanosystem was produced and characterized. The inclusion complex with the use of βCD was obtained in high yield and the lack of crystallinity observed by XRPD indicates inclusion complex formation. The inclusion complex TiF_4:βCD proved to be the only stable form and the

Table 2 – Surface microhardness (SMH) analysis of enamel blocks before and after pH-cycling, percentage of enamel surface change according to the treatments (means ± standard error) and cross-sectional microhardness (CMSH) analysis of enamel blocks according to the treatments (means ± standard error).

Treatments	SMH analysis			CMSH analysis
	SMH before	SMH after	% SML	ΔZ
Control	311.13 ± 4.66 ^{A}	113.91 ± 4.89 ^{B}	63.40 ± 1.45 ^{a}	750.7 ± 190.0^{a}
β-CD	319.57 ± 4.95 ^{A}	117.81 ± 4.08 ^{B}	63.13 ± 2.09 ^{a}	521.0 ± 319.7 ^{a,b}
TiF_4	325.89 ± 4.32 ^{A}	171.88 ± 6.5 ^{C}	47.07 ± 2.32 ^{b}	578.1 ± 236.2 ^{a,b}
TiF_4:β-CD	324.13 ± 3.76 ^{A}	135.81 ± 7.66 ^{B}	58.19 ± 2.16 ^{a}	483.8 ± 242.9 ^{b}

Means followed by distinct letters are statistically different ($p < 0.05$). Capital letters show difference between baseline and after pH-cycling and lower case letters show differences between the treatments after pH-cycling.

disappearance of the TiF_4 melting point peak provided evidence that a new supramolecular compound was formed with the characteristic of inclusion complexes. So, only this complex was further studied for inhibiting enamel demineralization capacity.

The TiF_4 solution is a low pH and non stable fluoridated compound. Despite its beneficial interactions with dental enamel, TiF_4 has not yet been used in clinical procedures as a fluoridated compound due to its instability in solution.[23,24] However, according to preview studies, TiF_4 reacts with enamel, forms an acid-resistant surface coating and increases the fluoride content of the dental enamel.[25,26] This coating is formed of TiO_2 and organometallic complexes (titanium and organic dental matrix).[9,26,27] The low TiF_4 pH favours the connection between the titanium and the oxygen from the phosphorus group found in hydroxyapatite.[10,25] The fluoride apatite decreases the solubility of the enamel and the formation of the titanium dioxide coating on the dental surface turns it more resistant to acid attack.[28,29] SEM analysis showed that the application of TiF_4 solution induces the formation of an acid-resistant titanium dioxide coating,[9,26,27] also presented in our study.

Furthermore, the TiF_4 efficiency may be related to the ability of replacing the calcium to the apatite structure or to the formation of titanium phosphate composites under the enamel surface.[30] In the present study, the TiF_4 group acted faster and more effectively in the enamel surface, protecting the enamel superficial layer from demineralization. This protection may be assigned to the formation of titanium dioxide coating as the presence of titanium on the enamel surface was confirmed by the SEM/EDX analysis.

The EDX analysis demonstrated the presence of numerous elements such as titanium, oxygen, calcium, carbon, sodium, magnesium, aluminium, phosphorus, chlorine, silicon and potassium. All elements except Titanium are usually found in sound or decayed enamel and dentine. Derise and Ritchey[31] showed that the microminerals including Fluorine, Iodine, Iron, Aluminium, Selenium, Manganese, Copper, Zinc, Strontium, Lead, and Cobalt are normally present in both sound and decayed enamel and dentine portions of permanent teeth. Neutron activation and atomic absorption were used in the analyses. Curzon and Losee[32] associated Aluminium, Barium, Copper, Lithium, and Zircronium with caries after the analysis of 208 teeth. Human and bovine enamel are similar in their mineral contents,[33,34] for this reason one can suppose that the aluminium observed in EDX spectrum is naturally present in teeth. However, aluminium is present in the enamel in a ppm-dimension and therefore below the detection limit of the EDX. It is more likely that the aluminium signal is an artefact of the SEM-sample-holders, since they are mostly made from aluminium.

The results demonstrate that the inclusion complex TiF_4:βCD was not able to protect effectively against surface mineral loss when assessed by the SMH analysis, since the mineral loss percentile of this group was significantly higher than the TiF_4 isolated group ($p < 0.05$) and statistically similar to the control group ($p > 0.05$). However, when the dental enamel was transversely assessed in the CMSH analysis (ΔZ), one could observe that the inclusion complex group offered more protection against mineral loss in the subsurface area ($p < 0.05$). Since the initial carious lesion is histopathologically characterized by subsurface mineral loss, the CSMH analysis can better represent the beginning of the carious process than SMH analysis.[35] Thus, the results for the inclusion complex (TiF_4:βCD) in CSMH analysis (ΔZ) showed good performance in reducing subsurface demineralization.

According to Loftsson et al.,[36] CDs possess the ability to improve pharmacokinetics and pharmacodynamics of complexed bioactive compounds in part by increasing the achievable concentration of the guest molecule in water, but also through improved stability to light and oxygen. Contrary to these beneficial features of CDs, the present study suggests that βCD may have a retarding effect on the action of TiF_4 in the superficial layer, slowing the effective penetration and overall depth of fluoride and titanium. Although the depth of Ti penetration and the concentration of this element in the enamel subsurface could not be determined, using EDX analysis of cross-sectional enamel samples, at least it was possible to observe the presence of Ti in the subsurface. This may explain the reduction of demineralization of the inclusion complex TiF_4:βCD to subsurface enamel when compared with control group. Another possibility is that titanium penetration appears to be greater in sound enamel than demineralized enamel.[2,4] This difference would be related to the bigger amount of water, carbonate and oxygen sources after the drying of sound enamel, improving the connection between the titanium and the oxygen present in the dental structure.

The solutions were applied once on the sound dental enamel by a dentist, such as in Magalhães et al.,[3] Chevitarese et al.[4] and Exterkate and ten Cate[2] who compared the use of TiF_4 on sound and demineralized enamel, concluding that the titanium penetration depth was higher in the sound enamel, as in the present study. This difference may be related to the larger amount of water, carbonate and oxygen sources after the drying

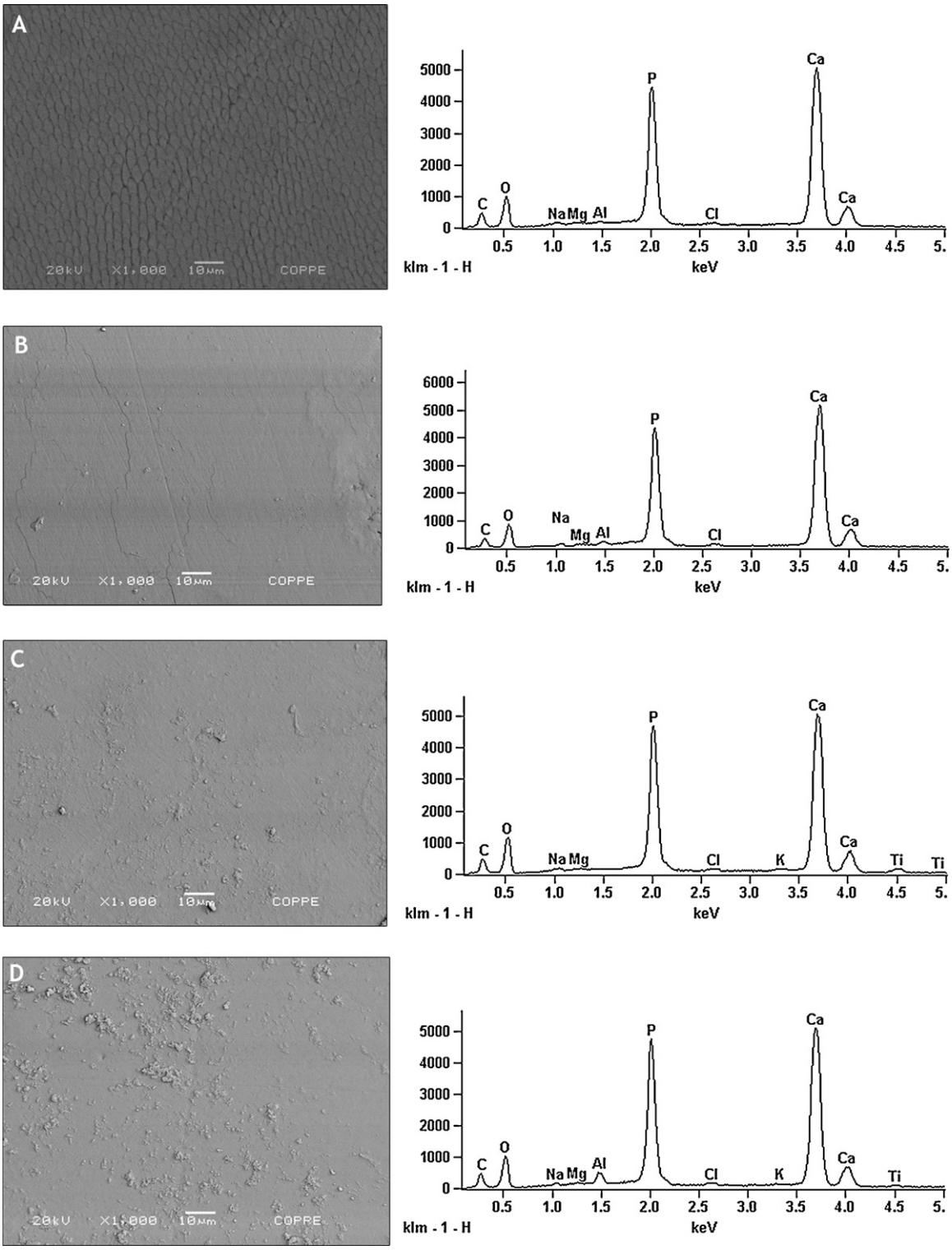

Fig. 1 – Surface SEM images of enamel samples after treatment and pH-cycling at 1000× (left) and EDS analysis (right). (A) Control, (B) βCD, (C) TiF_4 and (D) TiF_4:βCD. The (C) and (D) images show the presence of titanium in the EDS analysis.

of sound enamel, improving the connection between the titanium and the oxygen present in the dental structure.

The use of βCD to complex with TiF_4 proposed in this article presents the following improvements: an increase in the pH of TiF_4 nanosystems in solution, which enables its professional use and greater thermal stability. While the solution containing the inclusion complex TiF_4:βCD did not prevent the demineralization of enamel surface, it was the best at inhibiting the subsurface demineralization of enamel. For this reason, the application of the inclusion complex may be a

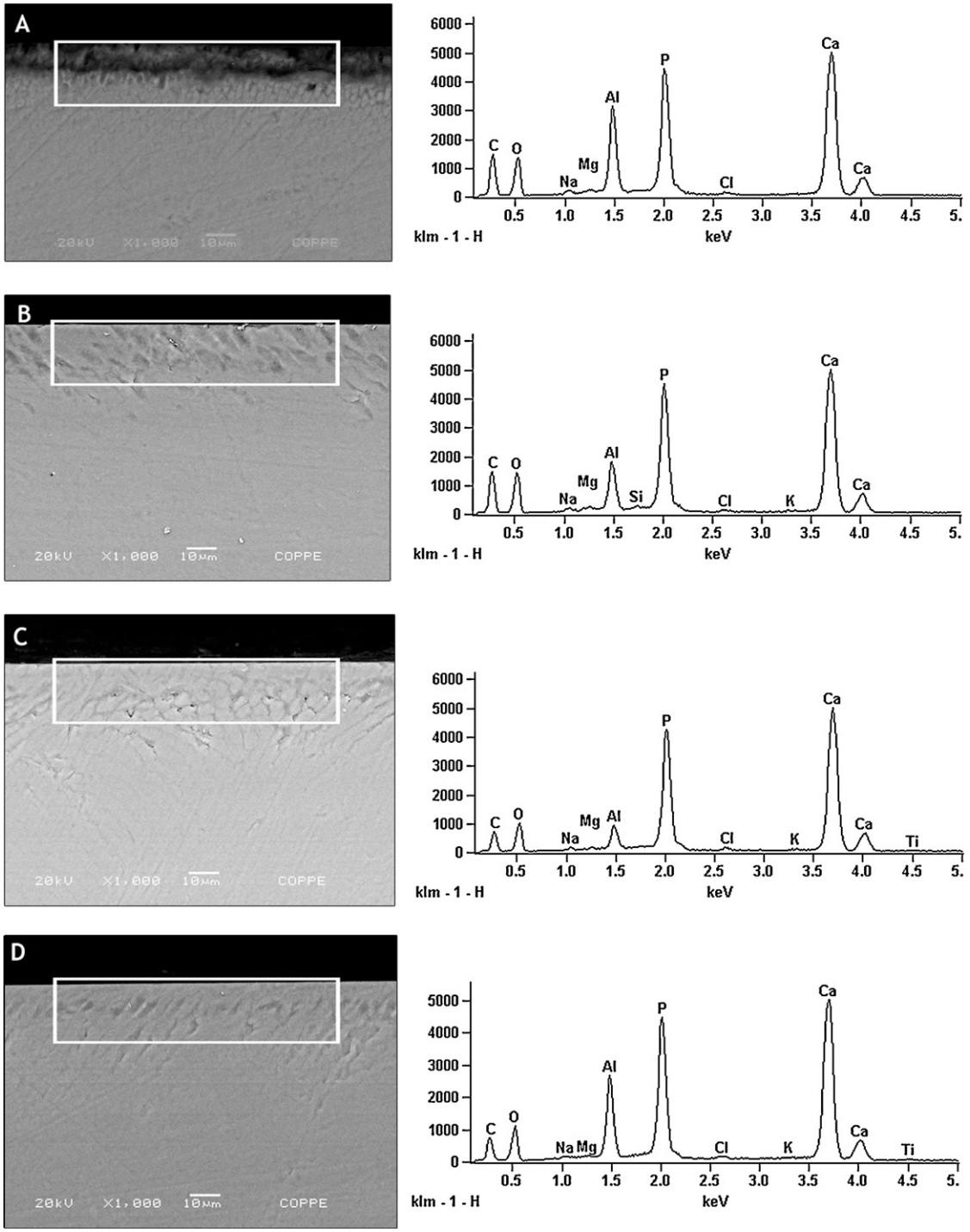

Fig. 2 – Cross-sectional SEM images of enamel samples after treatment and pH-cycling at 1000× (left) and EDS analysis (right). (A) Control, (B) βCD, (C) TiF_4 and (D) TiF_4:βCD. The (C) and (D) images show the presence of titanium in the EDS analysis.

new alternative to TiF_4 stabilization in order to avoid enamel demineralization.

5. Conclusion

A new inclusion complex of TiF_4 with βCD was demonstrated to be stable in solution and was able to reduced the subsurface demineralization of enamel. In order to transition this new complex into a clinical setting, additional studies need to be performed to determine conditions optimal complex formation and application protocols must be developed, including *in situ* studies.

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Competing interests

None declared.

Ethical approval

Not required.

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