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Fluoride and phosphate release from fluoride varnishes supplemented with nano-sized sodium trimetaphosphate

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ABSTRACT

Purpose: The addition of sodium trimetaphosphate (TMP) to fluoride (F) varnishes has been shown to be effective in controlling dental caries and erosive tooth wear. This study evaluated the amount of F and phosphate (P) released from fluoride varnishes containing micrometric or nano-sized sodium trimetaphosphate (TMPmicro or TMPnano, respectively).

Methods: The experimental varnishes included a placebo formulation (no F or TMP), 2.5% NaF, 5% NaF, 5% NaF + 5% TMPmicro, 5% NaF + 5% TMPnano, 5%NaF + 2.5% TMPnano, and commercial varnish (Duraphat, 5% NaF). Varnishes were applied on polyester sheets (n=8/group) and alternately immersed in remineralizing or demineralizing solutions at 30, 60, 90, 120, 180, 240, 300, 360, 420, 540, 600, 720, 780, 960, 1200 and 1440 min after first immersion. F and P release were analyzed with an ion-selective electrode and colorimetrically, respectively. Data were analyzed by 2-way, repeated measures ANOVA and Student-Newman-Keuls' test ($p<0.05$).

Results: Overall, varnishes containing TMPnano released significantly higher amounts of F in comparison with TMPmicro. Regarding the cumulative release of P, the varnishes containing TMP showed a constant increase up to 12 h.

Conclusions: It was concluded that F release was significantly increased in TMP-containing formulations, and that the nanosized TMP further enhanced such effects, without affecting phosphate release.

Key-words: *Fluorine; Polyphosphates; Sodium Fluoride; Nanoparticles.*

INTRODUCTION

Fluoride varnishes are professionally applied vehicles considered as slow fluoride-release products, due to their ability to adhere to tooth surfaces and to release fluoride to the oral environment for prolonged periods of time. The advantages of fluoride varnishes regarding ease of application, safety, patient's acceptability, and clinical efficacy are the main reasons for their widespread use in individuals of all ages[1].

In vitro and *in situ* data have shown that the addition of sodium trimetaphosphate (TMP) to fluoridated varnishes promote a synergistic effect on enamel remineralization[2,3], as well as on the reduction of enamel demineralization[4] and erosive wear[5,6]. Such effects were shown to be further enhanced by the use of nano-sized TMP, both on enamel remineralization and against erosive challenges[7,8].

To better understand the mechanisms of action of TMP-containing fluoridated varnishes, especially containing nano-sized particles, it is essential to determine the pattern of fluoride and TMP release from the varnishes over time. It has been previously reported that the amount of fluoride released from commercial varnishes are influenced by several factors, including the immersion medium[9] and type of resin used in the formulation[10,11]. Regarding products containing phosphate salts, conflicting evidence is available depending on the type of phosphate, in studies showing that varnishes containing calcium glycerophosphate[12] or TMP[13] released higher and lower amounts of fluoride, respectively, in comparison with varnishes without any phosphate added.

The above-mentioned variables that influence fluoride release from varnishes, the greater reactivity of nano-sized particles compared with conventional (micrometric) ones, and the need to assess the pattern of fluoride release from varnishes in protocols that better resemble clinical conditions regarding intraoral pH fluctuations motivated the present investigation. Thus, the aim of the study was to assess pattern of fluoride and phosphate release from varnishes containing micrometric or nano-sized TMP, in a pH-cycling model.

The present work provided data on the behavior of varnishes against changes in pH that occur in the mouth in a period of 24 hours. This would be useful to help ex-plain the mechanism of action of varnishes supplemented or not with TMP against acid challenges and remineralization processes that occur in the formation of initial caries lesions and dental erosion.

MATERIAL AND METHODS

Experimental design

The varnishes contained the same basic formulation, differing with respect to the concentrations of fluoride and TMP as follows: placebo (no fluoride or TMP), 2.5% NaF, 5% NaF, 5% NaF + 5% micrometric TMP, 5% NaF + 5% nano-sized TMP, 5%NaF + 2.5% nano-sized TMP, and a commercial varnish (Duraphat, 5% NaF), hereafter abbreviated as PLA, 2.5%NaF, 5%NaF, 5%NaF/5%TMPmicro, 5%NaF/5%TMPnano, 5%NaF/2.5%TMPnano, and Duraphat, respectively. The total fluoride concentration in the varnishes was measured prior to the beginning of the study. Following, a thin layer of varnish was applied on polyester sheets (n=8/group) and subsequently immersed in remineralizing (RE) or demineralizing (DE) solutions (3 mL) in the following sequence: 30 (RE), 60 (RE), 90 (DE), 120 (DE), 180 (RE), 240 (RE), 300 (DE), 360 (RE), 420 (DE), 540 (RE), 600 (DE), 720 (RE), 780 (DE), 960 (RE), 1200 (RE) and 1440 (RE) minutes after first immersion. Fluoride and phosphate were analyzed with an ion-selective electrode and colorimetrically, respectively.

Varnish formulation and fluoride assessment

The experimental varnishes were manufactured by SS White Dental Products (SS White Dental Products, Rio de Janeiro, Brazil) and contained the following components: colophony, ethyl cellulose, tolu balsam, beeswax, toluene sulfonamide, vanillin, saccharin and ethanol. Fluoride concentrations were 0%, 2.5% and 5% of NaF (Merck, Darmstadt, Germany), with or without the addition TMP (Aldrich Chemistry, China) at 5% (micrometric or nanozised) or 2.5% (nanozised). A varnish without F and TMP was also prepared (PLA). Fluoride concentrations in the varnishes were determined according to the protocol described by Shen and Autio-Gold (2002)[10] and Manarelli et al. (2013)[5]. Two samples of 0.5 mL were collected from the water phase and buffered with 0.5 mL of TISAB II.

Assessment of fluoride and TMP release from the varnishes

A thin layer of each varnish was applied on each side of polyester sheets (0.5 × 120 mm) (K Dent, Quimidrol, Brazil), which were weighed before and after the varnish application, providing information on the amount of varnish applied (n=8/group)[13]. Sheets were then placed inside polystyrene vials containing 3 mL of a remineralizing solution (1.5 mmol/L Ca, 0.9 mmol/L P, 0.15 mol/L KCl in 0.02 mol/L cacodylate buffer, 0.4 mL F, pH 7.0) or 3 mL a of demineralizing solution (2.0 mmol/L Ca and P in 0.075 mol/L acetate buffer, 0.45 mL F, pH 4.7) unstirred, at 37 ºC, allowing the varnish to be completely immersed in the solution. Each polyester sheet was placed in polystyrene vials in the following sequence: 30 (RE), 60 (RE), 90 (DES), 120 (DES), 180 (RE), 240 (RE), 300 (DES), 360 (RE), 420 (DES), 540 (RE), 600 (DES), 720 (RE), 780 (DES), 960 (RE), 1200 (RE) and 1440 (RE) minutes after first immersion. Fluoride released from all varnishes at each time interval was determined as previously described, calibrated with standards containing 0.5 to 16.0 µg F/mL. Two samples of 1 mL were collected from of each polystyrene vial and buffered with 1 mL of TISAB II[13].

The amount of phosphorus (P) released was determined according to Anderson et al. (1977)[14]. For this, 40 µL of 0.05 mol/L sulfuric acid and 40 µL of 1% periodic acid were added to 200 µL of the DE and RE solutions, and the resulting mixture was kept in boiling bath for 1 h. After cooling, 160 µL of deionized water was added, and an aliquot of 55 µL was transferred to a 96-well polystyrene plate (Costar, Tewksbury, MA, USA). Afterwards, 10 µL of 8% sodium sulfite and 5 µL of 1% sodium molibdate were added. After homogenization, 5 µL of 1% hydroquinone was added, and the plate was kept at 37° C during 30 min. Following, the volume of each well was adjusted to 250 µL with deionized water. Next, the absorbance readings were recorded at 640 nm by using a plate reader (PowerWave 340, Biotek, and Winooski, VT, USA). The amount of P in the DE and RE solutions was then subtracted from that originally present in the DE and RE solutions, allowing to calculate the amount of TMP present in the solutions.

Statistical analysis

Statistical analyzes were performed using the SigmaPlot (version 12.0), the level of statistical significance was established at 5% (SigmaPlot, Systat Software Incorporation, San Jose, USA). Data of fluoride and phosphate release showed normal distribution (Shapiro-Wilk test). The cumulative release value was calculated over the 24-hour period. Data obtained on the release of fluoride and phosphate in the DE and RE solutions as a function of time and varnish were analyzed by 2-way, repeated measures ANOVA and Student-Newman-Keuls' test ($p<0.05$). In order to simplify the interpretation of the data, 0.5 h, 2 h, 6 h, 12 h and 24 h were chosen to present the results.

RESULTS

Mean (SD) fluoride concentrations (µg F/g) in the varnishes were 434 (34), 10758 (302), 21379 (708), 20154 (327), 20400 (262), 19828 (317) and 23703 (1748), respectively for PLA, 2.5%NaF, 5%NaF, 5%NaF/5%TMPmicro, 5%NaF/2.5%TMPnano, 5%NaF/5%TMPnano and Duraphat.

Figure 1 shows the time-course fluoride release from the varnishes into remineralizing and demineralizing solutions determined at 0.5, 2, 6, 12 and 24 h after immersion into the solutions (Fig. 1A), as well as the cumulative release over time (Fig. 1B). The highest amount of fluoride was released by Duraphat. Also, a dose-response relationship was observed between the fluoride content in the test varnishes without TMP (PLA, 2.5%NaF and 5%NaF) and the amount of fluoride released. Regarding the TMP-containing varnishes, an exponential cumulative fluoride release was observed up to 6 h, reaching a plateau afterwards.

Overall, the amount of fluoride released from the varnishes increased when immersed in demineralizing solutions (Fig. 1A). At 2h (demineralizing solution), F re-lease from varnishes containing TMPnano was significantly higher when compared with 5%NaF and 5%NaF/5%TMPmicro ($p < 0.05$). At 6h (remineralizing solution), no significant differences were observed among the TMP-containing products, regardless of the particle size (Table 1). As for the cumulative release (Fig. 1B), varnishes containing TMPnano released significantly higher amount of fluoride in comparison with TMPmicro, except for the initial release (0.5 h).

As for phosphate release from the varnishes (Figure 2), a less defined pattern was observed when considering the release at each time point (Fig. 2A). On the other hand, the overall trend seen for the cumulative phosphate release (Fig. 2B) from the TMP-containing varnishes showed a constant increase up to 12 h, becoming less marked afterwards. For the varnishes without TMP, however, phosphate release remained fairly constant at low levels. Cumulative phosphate release from the TMP-containing varnishes was significantly higher than the other products from at 6 h and afterwards (Table 1), without significant differences among the varnishes supplemented with TMP, regardless of the particle size

Table 1. Fluoride released from the varnishes at each individual time point and cumulative release as a function of time and varnish composition

Groups	µg F/cm²									
	Released					Cumulative				
	0.5 h	2 h	6 h	12 h	24 h	0.5 h	2 h	6 h	12 h	24 h
Placebo	3.4^{a,A} (0.6)	3.2^{a,A} (0.6)	3.0^{a,A} (0.6)	3.4^{a,A} (0.7)	3.4^{a,A} (0.7)	3.4^{a,A} (0.7)	12.3^{a,B} (2.6)	24.0^{a,B,C} (5.1)	36.8^{a,C,D} (8.0)	50.0^{a,D} (10.7)
2.5% NaF	18.0^{b,A} (4.2)	18.4^{b,A} (3.0)	23.7^{b,A} (8.8)	67.9^{b,B} (19.3)	31.7^{b,C} (3.1)	18.0^{b,A} (4.6)	60.3^{b,B} (8.2)	137.8^{b,C} (23.1)	295.1^{b,D} (53.4)	450.6^{b,E} (68.3)
5% NaF	30.9^{c,A} (6.8)	51.9^{c,B} (6.3)	45.8^{c,B} (12.6)	140.5^{c,C} (15.9)	54.4^{c,B} (4.2)	30.9^{b,c,A} (7.5)	123.4^{c,B} (14.3)	288.9^{c,C} (38.6)	603.1^{c,D} (74.5)	886.5^{c,E} (95.2)
5% NaF/ 5%Micro	44.0^{d,A} (4.6)	65.9^{c,B} (16.0)	85.9^{d,B,C} (18.9)	114.9^{c,C} (31.3)	63.5^{c,B} (16.4)	44.0^{c,A} (5.0)	174.7^{d,B} (24.8)	397.7^{d,C} (50.9)	743.7^{d,D} (113.1)	1,041.1^{d,E} (159.2)
5% NaF/ 5%Nano	37.4^{c,A} (8.6)	101.3^{d,B} (15.7)	115.0^{d,B} (26.2)	132.0^{c,B} (42.0)	103.2^{d,B} (30.8)	37.4^{b,c,A} (9.5)	231.5^{e,B} (38.9)	558.2^{e,C} (72.0)	1,028.0^{e,D} (137.7)	1,443.6^{e,E} (202.1)
5% NaF/ 2.5%Nano	57.4^{d,A} (13.3)	113.5^{d,B} (23.9)	99.3^{d,B} (28.4)	105.8^{c,B} (20.6)	92.6^{d,B} (12.4)	57.4^{c,A} (14.6)	273.3^{e,B} (39.1)	576.5^{e,C} (97.5)	1,022.6^{e,D} (126.9)	1,429.8^{e,E} (135.7)
Duraphat	525.7^{e,A} (35.0)	181.6^{e,B} (32.5)	94.3^{d,C} (15.8)	191.8^{d,B} (34.8)	218.7^{e,B} (18.1)	525.7^{d,A} (38.4)	1,075.5^{f,B} (105.5)	1,581.6^{f,C} (136.9)	2,430.6^{f,D} (161.1)	3,246.5^{f,E} (190.7)

Values are presented as mean (SD). Different lowercase letters show significant difference among groups within each column. Different uppercase superscript letters indicate difference among the times of analysis within the same row (two-way ANOVA, Student-Newman-Keuls' test, $n=8$, $p<0.05$).

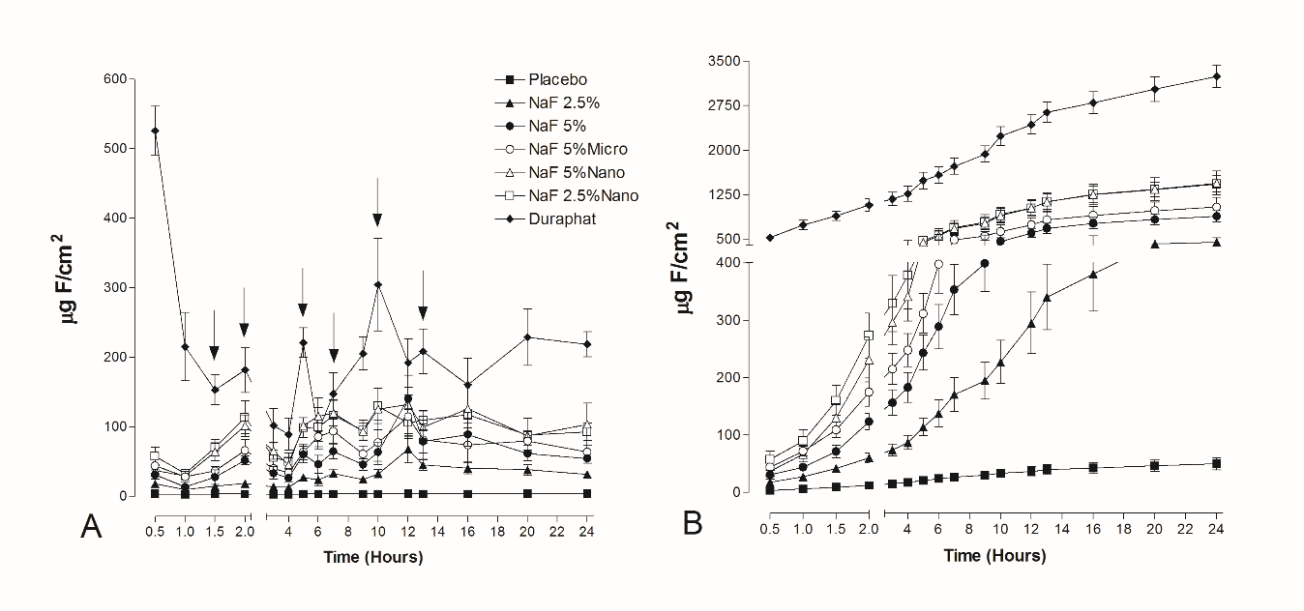

Figure 1. Time-course fluoride release from the varnishes into remineralizing and demineralizing solutions over 24 h. Vertical bars represent standard error of means, while the arrows indicate immersion in the demineralizing solution. A: fluoride release determined at each point. B: cumulative release.

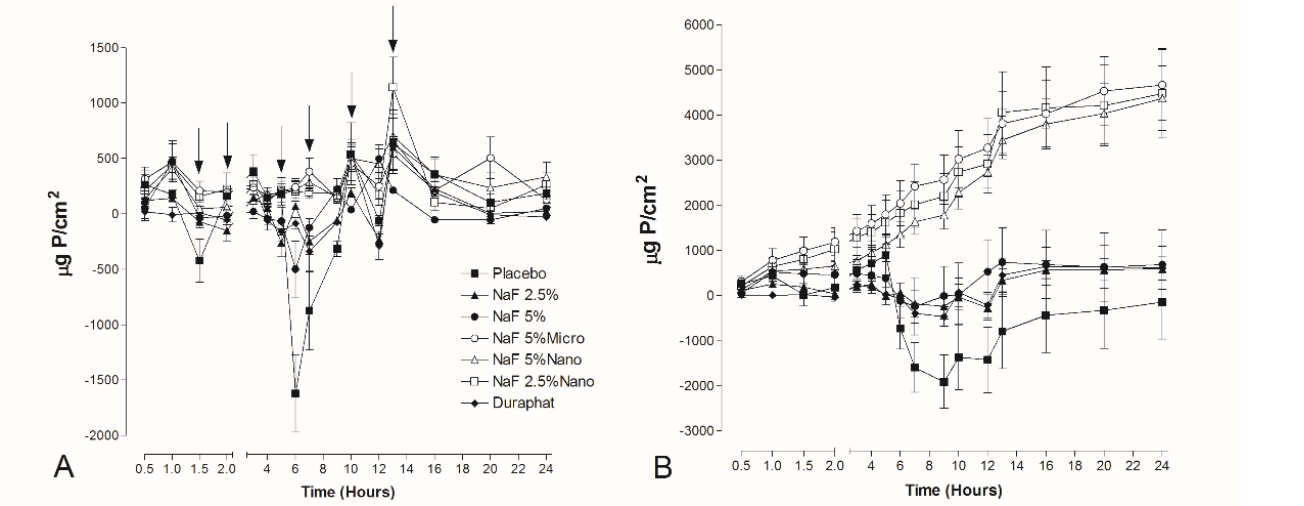

Figure 2. Time-course phosphorus release from the varnishes into remineralizing and demineralizing solutions over 24 h. Vertical bars represent standard error of means, while the arrows indicate immersion in the demineralizing solution. A: phosphorus release determined at each point. B: cumulative release.

DISCUSSION

A clear dose-response relationship was observed between F concentration in the test varnishes without TMP and the amount of F released into the media. Nonetheless, when comparing the two varnishes containing 5% NaF (test and commercial formulations), a significant difference was observed, since F release from Duraphat was ~1.4- to 17-fold higher than that from 5% NaF, depending on the time after first immersion. Given that both varnishes were manufactured with the same salt (NaF) and with the same natural resin (colophony), such differences are likely to be due to inherent properties of the carrier for NaF, including its viscosity, which was recently shown to influence the rate of F release from varnishes[15]. Similar data have been previously re-ported in studies assessing fluoride release into artificial saliva (instead of a pH-cycling regimen)[16,10,17], thus reinforcing the above-mentioned observations. However, it must be emphasized that the amount of fluoride released from different varnish formulations cannot be considered as a direct indicator of effect. A study con-ducted with the same varnishes as those used in the present study showed no differences between the two 5% NaF formulations (Duraphat and the experimental varnish) regarding their remineralizing effect[7]. Previous observations with different study protocols also support this observation[18,19,13,12,20].

The amount of fluoride released was also shown to be influenced by the addition of micrometric or nano-sized TMP. Despite all TMP-containing varnishes released significantly higher amounts of fluoride when compared with 5% NaF, the additional effect of 5% NaF/5% TMPmicro was ~17%, while the corresponding rate for both formulations containing nano-sized TMP was around 60%. Since the only difference between the varnishes supplemented with 5% TMP is the particle size of TMP (micrometric or nano-sized), one possible explanation for the higher fluoride release from the 5% NaF/5% TMPnano might be related to physico-chemical characteristics of this formulation. Considering a constant dissolution of colophony and other inactive ingredients of the formulations, the lower fluoride release from the 5% NaF (compared with the TMP-containing varnishes) is plausible when taking into account that carrier accounts for 95% of the total mass of the product, while the corresponding rate for the varnishes containing 5% NaF + 5% TMP is 90%. This could explain – at least in part – the lower fluoride release from the TMP-free varnish, as the higher proportion of carrier in the formulation would require a larger time for its dissolution, with a consequent impact on fluoride release from the varnish matrix. Regarding the differences in fluoride re-lease from varnishes containing TMPmicro and TMPnano, it is possible the higher particle size of TMPmicro would imply in a lower mobility of this salt from the varnish matrix, consequently affecting fluoride mobility.

It is noteworthy that the pattern observed for fluoride release from the 5% NaF/5% TMPmicro varnish in the present study was the opposite of that previously reported for the same formulation[13]. Considering that the varnishes used in the present study were produced by the same manufacturer and with the same ingredients as in the study conducted by Manarelli et al. (2016)[13], the different protocols for fluoride release is believed to be the main responsible for the discrepant results. While in the above-mentioned study fluoride release was assessed by immersion of the varnishes into artificial saliva solutions at neutral pH, the present protocol used a pH-cycling model (alternating from pH 7.0 to 4.7), in order to mimic pH fluctuations occurring in the oral cavity after varnish application. Furthermore, a previous study showed that fluoride release from varnishes under acidic conditions during 5 min (exposure to citric acid) was significantly higher than the corresponding release in artificial saliva during 30 min[9], what is in line with the release observed when the varnishes were immersed in the demineralizing solutions in the present study (Table 1 and Figure 1). All the above taken together suggest that the choice of a protocol for fluoride release from varnish formulations may have important implications related to the patterns observed, as discussed below.

In addition to varnish composition and TMP particle size, time was shown to also influence the pattern of fluoride release from the varnishes. The amount of fluoride re-leased in the first 30 minutes from experimental varnishes was very low, with values not significantly different among the 5% NaF experimental varnishes, regardless of the addition of TMP. Significant differences among the TMP-containing

varnishes first were detected at 2 h (considering cumulative fluoride release, Table 1) and the pattern of release exponentially rose up to 6 hours, before reaching a plateau. These results suggest that contact time between the varnishes and the tooth surfaces is paramount for optimizing the protective and/or therapeutic effects of varnishes[21] what has clinical implications regarding the professional recommendations and patient compliance. Nonetheless, taking into account the limitations inherent to a short-term, *in vitro* protocol, the study of fluoride release from these formulations under in vivo conditions could provide important data regarding the resulting fluoride levels in some biomarkers of exposure, including saliva, dental biofilm and biofilm fluid.

Regarding phosphate release from the varnishes, two aspects deserve comment. Firstly, despite the addition of nano-sized TMP significantly enhanced fluoride release when compared with TMPmicro, such effects were not observed for phosphate release. Considering both varnishes containing 5% TMP (for comparison purposes), the lack of significant differences in phosphate release seem to confirm the above-mentioned hypothesis that the rate of dissolution of the varnish matrix is a determining aspect for the release of the active ingredients of the formulations. Following this rationale, the amount of phosphate released from both varnishes containing 5% TMP would, there-fore, not be influenced by the particle size, what is in accordance with the present data. The second aspect is related to the lack of significant differences between the two varnishes supplemented with TMPnano. Due to the two-fold difference in TMP concentrations between the two formulations, it would be expected that the differences in the resulting phosphate release from the formulations would, therefore, be around 100%. The reasons for such a trend are not apparent, and since this is the first study assessing phosphate release from TMP-containing fluoridated varnishes, any hypothesis raised on these results would be too speculative, so that future studies with different research protocols would be instructive.

Taking the results of fluoride and phosphate release from the varnishes together, along with data on the effects of these formulations on the remineralization of artificial caries lesions and on the protection of enamel against erosive challenges[7,8], it seems logical that the constant release of TMP and the enhanced fluoride release from the varnishes supplemented with TMPnano are the reasons for the higher preventive and therapeutic effects of these products. Further studies, however, are still required in order to confirm the additional effect of TMPnano on the dynamics of dental caries and erosion, especially employing different *in vitro* models and subsequent *in situ* protocols that better resemble clinical conditions.

CONCLUSIONS

The results show that the supplementation of fluoride varnishes with TMP significantly increases the amount of fluoride released from the formulations, with an additional effect achieved by the use of nano-sized particles. Phosphate release from the TMP-containing varnishes was not affected by particle size.

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CONFLICT OF INTERESTS

A patent was requested for a product used in the study, by the National Institute of Industrial Property - INPI/SP, on 10/17/2014 under number BR 10 2014 025902 3.

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