# Fluoride Release from Fluoride Varnishes under Acidic Conditions

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***Objective:*** *The aim was to investigate the in vitro fluoride release from fluoride varnishes under acidic conditions.* ***Study design:*** *Poly(methyl methacrylate) blocks (Perspex, n=3 per group) were painted with 80±5 mg fluoride varnish (n=10) and placed into artificial saliva for 30min. Then, blocks were placed into either 1% citric acid (pH 2.27) or 0.3% citric acid (pH 3.75) solutions (n=3 per solution and varnish) for 30min with the solutions being replaced every 5min. Saliva and acid solutions were analyzed for fluoride content. Data were analyzed using three-way ANOVA (varnish, solution, time).* ***Results:*** *The three-way interaction was significant (p<0.0001). Fluoride release and release patterns varied considerably between varnishes. Fluoride release in saliva varied by a factor of more than 10 between varnishes. Some varnishes (CavityShield, Nupro, ProFluorid, Vanish) showed higher fluoride release in saliva than during the first 5min of acid exposure, whereas other varnishes (Acclean, Enamel-Pro, MI Varnish, Vella) showed the opposite behavior. There was little difference between acidic solutions.* ***Conclusions:*** *Fluoride release from fluoride varnishes varies considerably and also depends on the dissolution medium. Bearing in mind the limitations of laboratory research, the consumption of acidic drinks after fluoride varnish application should be avoided to optimize the benefit/risk ratio.*

***Keywords:*** *Fluoride varnish, fluoride release, dental erosion*

## INTRODUCTION

In the USA, fluoride varnishes (FV) were approved by the Food and Drug Administration (FDA) for the use as cavity liners or for the treatment of dentin hypersensitivity in 1994. However, FV were and are still not approved for the prevention of dental caries. Hence, dental professionals, and especially pediatric dentists, also use FV 'off-label' as a topical anti-caries fluoride agent,[1] although none of the commercially available FV in the USA are marketed as such. In recent years, more FV have been marketed—32 were identified based on a personal internet search as of March 2013, in comparison to only three in 2000.[2] This surge can be largely ascribed to evidence-based clinical recommendations on professionally applied topical fluoride published by the American Dental Association Council on Scientific Affairs in 2006.[3] It was concluded that "fluoride varnish applied every six months is effective in preventing caries in the primary and permanent dentition of children and adolescents", thereby paving the way for a more widespread use of FV by pediatric dentists. Indeed, the American Academy of Pediatric Dentistry recommends the application of fluoride varnish for children with increased caries risk as part of their 'Guideline on Fluoride Therapy'.[4]

Most research on FV has focused on determining their efficacy—either in vivo by means of caries reduction or via secondary endpoints in vitro, such as fluoride release into the surrounding medium,[5,6,7,8,9] fluoride uptake by dental hard tissues,[10,11,12,13] or remineralization of early caries lesion.[14] [15,16] A recent review[17] concluded that FV do not pose a risk to fluoride toxicity and its adverse effects, such as dental fluorosis. However, little attention has been paid to optimize the benefit/risk ratio of FV, as the majority of fluoride released into saliva will undoubtedly be ingested during the post-application phase and depending not only on the subject's level of compliance but also on the type of FV applied.

Post-application instructions for FV do almost uniformly suggest children to stay on a soft diet and to avoid hot beverages for at least 4 h. Poor compliance could potentially result in the removal/dissolution of applied FV which would consequently lower the benefit and unnecessarily increase the risk of fluoride toxicity. However, apart from the aforementioned restrictions, no manufacturer provides instructions about the type of beverages that can be consumed. Considering the widespread consumption of acidic soft

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drinks, such as sodas, fruit juices and ice teas, and especially among children and adolescents,[18] a better understanding of fluoride release from FV under acidic conditions is warranted. Hence, the aim of the present study was to investigate the *in vitro* fluoride release from fluoride varnishes under mild and strong acidic conditions.

## MATERIALS AND METHOD

Details about the tested ten FV and their respective dietary instructions relating to beverages can be found in Table 1. All tested FV claimed to contain 5 % sodium fluoride.

Poly(methyl methacrylate) blocks (Perspex; 1 × 1 × 0.75 $in^3$) were used as the study substrates. Blocks were cleaned with deionized water and FV was applied to one of the 1 × 1 $in^2$ smooth surfaces on each block. For this, the protective foil from the individual FV dose was removed and the FV mixed using the manufacturer's applicator (typically a microbrush) for at least 10 s to homogenize the FV, as sedimentation of sodium fluoride and phase separation may have occurred during storage. Subsequently, approx. 80 ± 5 mg FV were evenly applied to each Perspex block using the manufacturer's applicator (typically a microbrush). The amount of FV applied was recorded. The experiment was conducted in triplicate; i.e. six FV-painted Perspex blocks were prepared for each FV (three each for mild and strong acidic conditions).

Immediately after FV application, blocks were placed individually into 60 ml plastic containers. 30 ml of artificial saliva (1.45 mM $CaCl_2 \cdot \times 2H_2O$; 5.4 mM $KH_2PO_4$; 14.9 mM KCl; 28.4 mM NaCl; 2.2 g/l porcine gastric mucin; pH 7.0, adjusted using KOH) were poured carefully into each container, fully submerging the block. The container was then placed onto an orbital shaker set at 100 rpm for 30 min. After 30 min, saliva was decanted into a separate, pre-weighed container and the weight of saliva determined. Immediately afterwards, three of the six blocks per FV were exposed to a mild acidic solution (0.3 % w/w anhydrous citric acid in deionized water, pH 3.75, adjusted using NaOH), whereas the remaining three blocks were exposed to a strong acidic solution (1 % w/w anhydrous citric acid in deionized water, pH 2.27, unadjusted). Acidic solutions were poured carefully into each container, fully submerging the block. The containers were then placed onto an orbital shaker set at 100 rpm for 30 min. Every 5 min, the solutions were decanted into a separate, pre-weighed container and the weight of solution determined. Solutions were then renewed until all blocks were exposed to either the mild or strong acidic solution for 30 min. The rationale for the chosen study design was to mimic the slow *in vivo* consumption of a soft drink.

Aliquots of the collected saliva and mild and strong acidic solutions were mixed 1:1 with TISAB II and analyzed for fluoride in comparison to a similarly prepared standard curve. Fluoride concentrations were analyzed using a fluoride ion-sensitive electrode. Fluoride release data were normalized for the amount of FV applied.

The statistical analyses were done using a mixed-model ANOVA with a random effect to correlate data within a specimen and allowed each varnish to have a different variance, and multiple-comparisons adjustment to control the overall significance level at 5%. Analyses were done using natural-logarithm transformed data.

## RESULTS

The three-way interaction between FV, solution and exposure time was significant ($p<.0001$). Thus, the comparisons are shown for each treatment combination. Tables 2 (mild) and 3 (strong acidic challenge) present the mean, normalized fluoride release data and the results of the statistical analysis.

Fluoride release into saliva (30 min) varied considerably between FV and by a factor of more than 10—e.g. from 20.8 (Acclean) to 3457.1 (Nupro) µg fluoride/g fluoride varnish (strong acidic challenge groups). Although of statistical significance, differences in fluoride release between acids but within FV were relatively small

**Table 1.** Tested fluoride varnishes and dietary instructions relating to drinks

| Fluoride varnish | Manufacturer | Lot/batch number; expiry date | Dietary instructions relating to beverages |
|---|---|---|---|
| Acclean | Henry Schein | 38605; Nov 2013 | …only…drink cold liquids for four hours after treatment. |
| Butler | Sunstar Americas | T314HE; Nov 2013 | Beverages, mouthwashes or rinses containing alcohol should also be avoided for at least 4-6 hours after application. |
| CavityShield | 3M ESPE | K11522W; Jun 2013 | Avoid hot drinks and products containing alcohol…during the treatment period (4-6 hours). |
| Enamel Pro | Premier Dental | 38885; Dec 2013 | …avoid hot beverages and products containing alcohol during the treatment period (4-6 hours). |
| MI Varnish | GC America | 1109121; Sep 2014 | …avoid…products containing alcohol…while the MI Varnish is on the teeth (4 hours min.). |
| Nupro | Dentsply | 120131; Sep 2013 | …remain on a soft-food diet…for at least 2 hours after treatment (no drink related instructions). |
| Patterson | Patterson | 38656; Nov 2013 | …only…drink cold liquids for two hours after treatment. |
| ProFluorid | Voco | 1145425; Mar 2013 | …avoid…alcohol…for the next 4 hours after application. |
| Vanish | 3M ESPE | 14690J3K; Apr 2013 | Avoid hot drinks and products containing alcohol…during the treatment period (4-6 hours). |
| Vella | Preventive Technologies | 38286; Sep 2013 | …only…drink cold liquids…during the treatment period (four hours). |

**Table 2.** Mean fluoride release (µg fluoride/g fluoride varnish) and results of statistical analysis for the mild erosive challenge group

| Varnish | Saliva | | 5 min | | 10 min | | 15 min | | 20 min | | 25 min | | 30 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acclean | 17.9 | e B | **43.9** | f A | 14.7 | f B | 4.3 | f C | 2.4 | e CD | 2.3 | e CD | 1.6 | e D |
| Butler | 486.7 | bc A | 457.8 | bc A | 333.1 | bc A | 354.3 | b A | 295.2 | a A | 240.1 | a A | **220.2** | a A |
| CavityShield | 231.1 | cd A | 98.5 | ef B | 49.4 | e BC | 24.3 | e CD | 25.0 | d CD | 13.4 | d D | 13.7 | cd D |
| Enamel Pro | 1911.7 | a A | 2104.0 | a A | 1234.9 | a B | 743.3 | a C | 528.6 | a D | 392.8 | a DE | 330.9 | a E |
| MI Varnish | 157.2 | d B | 328.1 | c A | 133.1 | d B | 40.1 | e C | 21.5 | d CD | 22.1 | cd CD | 13.4 | cd D |
| Nupro | 2356.1 | a A | 710.8 | b B | 544.1 | b C | 471.0 | ab CD | 415.7 | a D | 409.1 | a D | 403.1 | a D |
| Patterson | 396.5 | bcd A | 337.7 | bcd A | 80.7 | de B | 39.1 | de BC | 21.5 | cd BC | 17.8 | cd C | 16.4 | cd C |
| ProFluorid | 595.1 | b A | 319.0 | c B | 192.5 | cd C | 132.5 | c CD | 92.2 | b D | 58.7 | b E | 49.2 | b E |
| Vanish | 378.7 | bc A | 136.8 | de B | 38.0 | e C | 20.4 | e CD | 18.3 | d CD | 11.7 | d D | 9.6 | d D |
| Vella | 159.8 | d BC | 382.6 | bc A | 222.0 AB | cd | 97.4 | cd CD | 65.4 | bc DE | 40.0 | bc EF | 24.8 | bc F |

Standard deviations have been omitted for better clarity but are shown for Pro-Fluorid and MI Varnish in Figures 1 and 2 respectively.

Statistically significant differences between fluoride varnishes within saliva/acid exposure times are highlighted by different small letters, differences between saliva/acid exposure times within each fluoride varnish by different capital letters.

Statistically significant differences between mild and strong erosive challenges (compare to Table 3) within fluoride varnishes and saliva/acid exposure times are highlighted in bold.

**Table 3.** Mean fluoride release (µg fluoride/g fluoride varnish) and results of statistical analysis for the strong erosive challenge

| Varnish | Saliva | | 5 min | | 10 min | | 15 min | | 20 min | | 25 min | | 30 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acclean | 20.8 | e B | **445.1** A | bc | 16.7 | c B | 7.8 | e C | 4.1 | e CD | 2.7 | e DE | 1.7 | f E |
| Butler | 795.4 abcd A | | 113.4 AB | cd | 58.4 | bc B | 102.0 | bcd AB | 48.6 | bcd B | 46.5 | bcd B | **32.9** bcde B | |
| CavityShield | 199.4 | d A | 73.3 | d B | 22.3 | c C | 13.8 | de C | 12.9 | d C | 10.1 | d C | 7.5 C | de |
| Enamel Pro | 1514.6 AB | b | 1916.4 | a A | 924.8 | a BC | 614.4 | a CD | 421.4 | a DE | 350.2 | a DE | 317.0 | a E |
| MI Varnish | 173.5 | d B | 412.7 A | bc | 145.4 | b BC | 70.4 | c CD | 36.4 | bc DE | 23.5 | cd EF | 14.2 cde F | |
| Nupro | 3457.1 | a A | 799.9 | b B | 598.3 | a BC | 499.7 | ab BC | 425.0 | a C | 381.1 | a C | 370.5 | a C |
| Patterson | 248.8 AB | d | 269.8 | c A | 126.5 | b BC | 88.6 | c CD | 59.6 | b DE | 43.0 | bc EF | 21.0 bcd F | |
| ProFluorid | 601.3 | c A | 288.7 | c B | 163.4 | b C | 92.3 | c D | 70.6 | b E | 52.0 | b F | 40.5 G | b |
| Vanish | 294.8 | d A | 99.2 | d B | 31.8 | c C | 18.0 | d CD | 17.9 | cd CD | 15.4 | d DE | 8.5 | e E |
| Vella | 164.7 AB | d | 315.7 | c A | 178.5 | b A | 75.3 | c BC | 58.1 | b CD | 45.5 | bc DE | 34.3 E | bc |

Standard deviations have been omitted for better clarity but are shown for Pro-Fluorid and MI Varnish in Figures 1 and 2 respectively.

Statistically significant differences between fluoride varnishes within saliva/acid exposure times are highlighted by different small letters, differences between saliva/acid exposure times within each fluoride varnish by different capital letters.

Statistically significant differences between mild and strong erosive challenges (compare to Table 2) within fluoride varnishes and saliva/acid exposure times are highlighted in bold.

and affected only two samples (Acclean 5 min; Butler 30 min). Overall, there were two patterns of fluoride release—one group of FV (CavityShield, Nupro, ProFluorid – Figure 1, Vanish) exhibited higher fluoride release in saliva than during the first 5 min of acid exposure and gradually declined thereafter. Other FV (Acclean, Enamel-Pro, MI Varnish – Figure 2, Vella) showed the opposite behavior. These FV released more fluoride during the first 5 min of acid exposure than during the 30 min of exposure to artificial saliva before showing a gradual decline. All FV showed a similar, gradual decline in fluoride release with continuing acid exposure.

**Figure 1.** Fluoride release from ProFluorid varnish – into artificial saliva (0-30 min), followed by erosive solutions (30-60 min) in 5-min intervals. Error bars denote standard deviations.

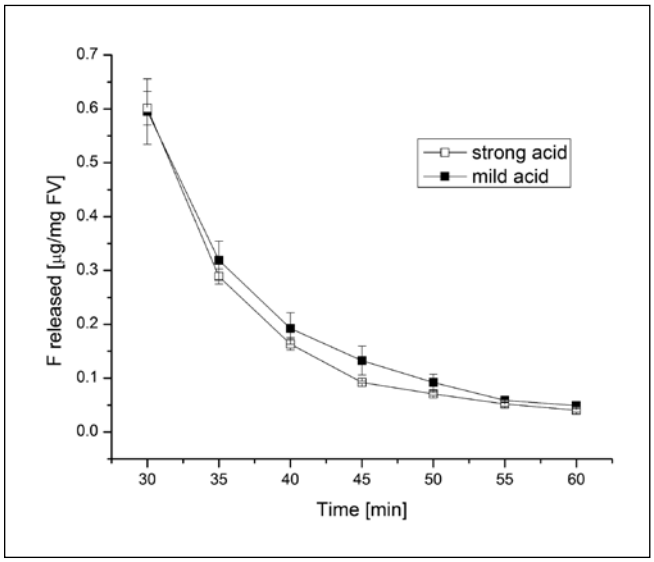
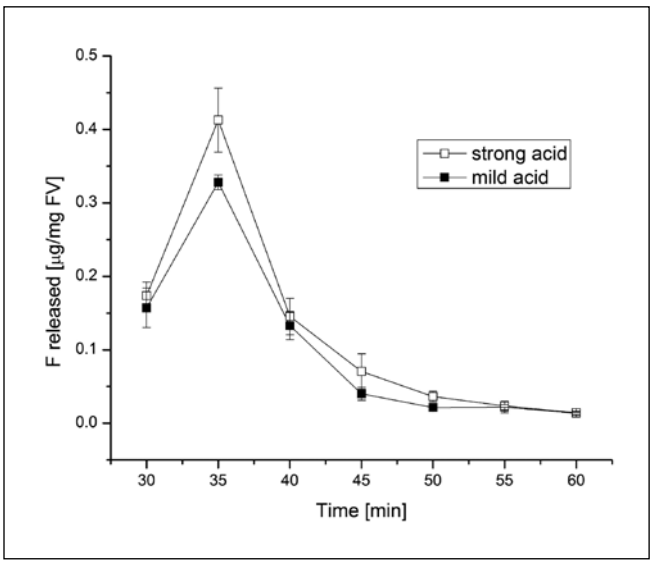



**Figure 2.** Fluoride release from MI varnish – into artificial saliva (0-30 min), followed by erosive solutions (30-60 min) in 5-min intervals. Error bars denote standard deviations.




## DISCUSSION

The present in vitro study has shown that FV do not only vary in their fluoride release into artificial saliva, which mirrors previous studies,[4,5,6,7,8] but also into acidic solutions, representative of soft drinks of either relatively low or high pH.[19] The experiment was designed to mimic the clinical scenario immediately following a FV application – 30 min of exposure to (artificial) saliva followed by the slow consumption of a soft drink over an equal period of time. The present results, once confirmed under more clinically relevant conditions, have direct impact on pediatric dentists and their post-treatment dietary instructions for their patients.

While it can be assumed that children will be compliant with the dietary instructions for several hours after a FV application, none of the instructions (Table 1) prevent children from consuming low pH non-alcoholic drinks. Based on the present study's findings, these drinks have the potential to "leach out" fluoride from FV and therefore decrease the benefit/risk ratio as the dissolved fluoride will be ingested with the soft drink and unnecessarily increases the subject's risk of fluoride toxicity. Present dietary instructions would need to be extended as they currently only highlight that children should refrain from hot or alcoholic beverages (which would dissolve the FV carrier material, although somewhat irrelevant for children) and stay on a soft diet (hard foods would abrade the FV). However, the present data showed inconsistencies as some FV showed considerably more release under acidic conditions than others (Tables 2 and 3, comparing 30 min saliva vs. first 5 min of acid exposure). Several FV were particularly prone to low pH fluoride loss and this was not related to their overall fluoride release into saliva. It can be speculated that inherent formulation differences are accountable for their different behaviors, but this does not necessarily explain the observed differences in fluoride release into saliva and acid between FV. As manufacturers are not required to disclose any formulation details other than a bare minimum in material safety data sheets, future research would need to be directed at dissembling formulations to examine which excipients are responsible for fluoride release at different pH values.

Dental erosion has been defined as the "physical result of a pathologic, chronic, localized, painless loss of dental hard tissue chemically etched away from the tooth surface by acid and/or chelation without bacterial involvement".[20] Considering the increasing prevalence of dental erosion in the USA,[2122] with the consumption of acidic soft drinks being the major contributing etiological factor,[8] more emphasis should be paid on nutrition education to not only limit the irreversible loss of tooth structure but also—as the present study has shown—to optimize the benefit/risk ratio of a topical fluoride treatment (e.g. varnish, gel, foam, rinse). Fluoride can be taken up by enamel in various forms, which can be, albeit crudely, divided into loosely- (e.g. as calcium fluoride) and structurally-bound fluoride (e.g. as fluoridated hydroxyapatite).[23] The former is the primary mode of fluoride uptake by enamel and dentin, acts as a labile reservoir of fluoride ions, and can serve as a source for the latter.[24] Indeed, such observations were made after FV application *in vivo*.[10] Fluoride applied at high concentrations, such as from FV, gels and foams, will primarily form non-stoichiometric calcium fluoride,[22] which is very labile and more soluble at low pH than in resting saliva.[25] Thus, the consumption of acidic soft drinks should be avoided immediately after the application of a professionally applied fluoride product.

The present study was a first attempt at gaining a better understanding of fluoride release from FV under acidic conditions *in vitro*. Care must be taken not to over-interpret data derived from laboratory studies as these only mimic the clinical situation. Future research is undoubtedly necessary to gain a better understanding of the topic—the effect of flow rate, temperature (soft drinks are often consumed cold), types of acids (e.g. citric, phosphoric) and the effect of dilution (drinks consumed with ice cubes) will have to be studied.

## CONCLUSIONS

The *in vitro* fluoride release from fluoride varnishes varies considerably and is also dependent on the dissolution medium. Several varnishes exhibited enhanced fluoride release under acidic conditions in comparison to exposure to artificial saliva. Bearing in mind the limitations of the present in vitro study, the consumption of acidic drinks after fluoride varnish application in children should be avoided to optimize the benefit/risk ratio. However, future, clinical research is required to confirm the present findings.

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