In Vitro Comparison of the Fluoride Ion Release from the First- and Second-Generation Fluoride Varnishes

Dagmara Piesiak-Panczyszyn 1,*, Adam Watras 2,3,*, Rafal J. Wiglusz 2 and Maciej Dobrzynski 3,*

1 Department of Conservative Dentistry with Endodontics, Wroclaw Medical University, 50-425 Wroclaw, Poland
2 Institute of Low Temperature and Structure Research, Polish Academy of Sciences, 50-422 Wroclaw, Poland; r.wiglusz@intibs.pl
3 Department of Pediatric Dentistry and Preclinical Dentistry, Wroclaw Medical University, 50-425 Wroclaw, Poland
* Correspondence: dagmara.piesiak-panczyszyn@umw.edu.pl (D.P.-P.); a.watras@intibs.pl (A.W.); maciej.dobrzynski@umw.edu.pl (M.D.)

Abstract: Fluoride varnishes, both the first and the second generations, are effective in inhibiting caries, especially in children and adolescents, by reducing it on average by 43% for permanent teeth and 37% for deciduous teeth. The aim of this study was to evaluate the dynamics of in vitro fluoride ion release from first- (Duraphat) and second-generation (MI Varnish and Embrace Varnish) fluoride varnishes and the impact of the type of varnish, the time from its application and the pH of the environment on this process. Materials and methods: The test material (90 specimens), prepared from extracted human teeth, were divided into nine groups of 10 specimens each. Measured amounts of the examined varnishes were applied onto specimens and the levels of fluoride release were assessed at the baseline and after 1, 2, 24, 48 and 168 h from the application with the use of an ion-specific electrode. The specimens were immersed into artificial saliva with pH adjusted to 4, 5 and 7. The highest cumulative release of fluoride was obtained by MI Varnish (11.52 ppm/mg), regardless of the pH of the environment, whereas the lowest released fluoride concentration was achieved by Embrace Varnish (4.82 ppm/mg). In the acidic environment, the release of fluoride was significantly higher than in the neutral environment for all investigated varnishes, with no change in the overall fluoride release profile and with maximum fluoride release in the first two hours after application. The findings of this study indicate that all examined fluoride varnishes released the maximum amount of fluoride within the first hours after application and that it was related to the acidity of the immersion medium.

Keywords: fluoride varnishes; fluoride ion release; fluoride release rate

1. Introduction

For almost 70 years, fluoride compounds have played an important role in preventing dental caries as the most effective method of fighting this disease. Their action is based on two mechanisms: pre-eruptive (endogenous) and post-eruptive (exogenous), whereby exogenous methods are now considered more effective and safer [1–6]. The post-eruptive effect, i.e., after the emergence of teeth in the mouth, consists in affecting the adhesion of the bacterial plaque and its metabolism, thus causing a bacteriostatic and/or bactericidal effect, inhibiting the demineralization process and supporting the remineralization of the enamel [1,3,7–11]. Fluoride varnishes used in the contact fluoridation method were approved in 1994 by the FDA (Food and Drug Administration), but as a medicine for tooth hypersensitivity. Over the years, they have become standard practice in dentistry, currently recommended by international dental organizations and associations, i.e., ADA (American Dental Association), AAPD (American Academy of Paediatric Dentistry), EAPD (European
Academy of Paediatric Dentistry) and the American Dental Association Council on Scientific Affairs [12–15]. Numerous meta-analyses [9,16–18] have shown their effectiveness in inhibiting caries, especially in children and adolescents, by reducing it on average by 43% for permanent teeth and 37% for milk teeth. The lack of continuation of caries prevention with the aid of administration of fluoride, according to Bravo et al. [19], resulted in an increase in caries activity.

The exact chemical composition of varnishes is not fully known because, according to the statement developed by OSHA, the so-called Hazard Communication Standard 29 CFR 1910.1200, only those ingredients that can be dangerous and life-threatening require documentation; the others are a trade secret [20]. Typically, each fluoride varnish consists of three basic elements. Two of them are resin (mastic, shellac or/and rosin, also called colophony), which is responsible for viscosity, elasticity and fluidity and thus extending the contact time with the tooth surface; and alcohol (usually ethanol), used as a solvent which evaporates after exposure to air and active agents [7,21]. In addition, in the so-called first generation of varnishes, there are fluoride salts, commonly known as fluorides, usually in the form of neutral or acidified 5% NaF containing 2.26% fluoride ions (22,600 ppm F) or 1% difluorosilane containing 0.1% fluoride ions (1000 ppm F). The second-generation varnishes are additionally enriched with calcium and phosphorus compounds in various forms, e.g., as so-called functionalized tricalcium phosphate (β-TCP), amorphous calcium phosphate (ACP), casein phosphopeptide amorphous calcium phosphate nanocomplex (CPP-ACP), calcium sodium phosphosilicate (Novamin) or xylitol-coated calcium phosphate (CXP) [22,23]. According to many researchers [22,24–30] these increase the bioavailability of fluoride and the rate of its release as well as improve oral retention and enhance the antibacterial effect. The aim of this study was to evaluate the dynamics of in vitro fluoride ion release from first- and second-generation fluoride varnishes and the impact of the type of varnish, the time from its application and the pH of the environment on this process. The extent to which factors influence the durability of the applied varnish, the amount of released fluoride ions over time and the duration of maintaining an elevated level of fluoride may alter the accepted treatment standards and contribute to enhancing the effectiveness of therapy.

2. Results

The average values of the varnish (the quantity of varnish in mg) used ranged from $7.9 \pm 0.2645$ mg to $8.2 \pm 0.1527$ mg, which corresponded to the average fluoride content in the range from $0.1249 \pm 0.0055$ mg for MI Varnish, $0.1512 \pm 0.0027$ mg for Duraphat and $0.1640 \pm 0.0065$ mg for Embrace Varnish.

Figure 1 graphically represents the cumulative release of fluoride for the three varnishes in three different pH (4, 5 and 7) environments, expressed in ppm. Within 168 h of the experiment, regardless of the pH of the environment, the highest amount of fluoride was released by MI Varnish, and the lowest level was released by Embrace Varnish. The cumulative release of fluoride for MI Varnish was $11.52$ ppm $F^-$ at pH = 4, $9.297$ ppm $F^-$ at pH = 5 and $6.470$ ppm $F^-$ at pH = 7. Duraphat achieved $9.753$ ppm $F^-$, $7.513$ ppm $F^-$ and $9.276$ ppm $F^-$, respectively; Embrace Varnish achieved $6.826$ ppm $F^-$, $5.724$ ppm $F^-$ and $4.821$ ppm $F^-$, respectively.

In most research groups, significantly higher values were obtained in acidic environments (pH = 4 and 5) as compared with the neutral environment. For MI Varnish, the release of fluoride in the environment with various acidities was different after the first hour; for Duraphat, after 2 h; and for Embrace Varnish, after 24 h. For all three varnishes tested, the highest increases in fluoride release were recorded during the first 2 hours of observation, with both MI Varnish and Embrace Varnish releasing, respectively, more than 50% and 60% of the total 168 h fluoride release during the first 2 h after application, and more than 90% and 85% after 24 h. Duraphat, on the other hand, during the same time, showed a slower release rate at an average level of 36% after 2 and 68% after 24 h. Based on the results of the variance analysis and post hoc tests, the cumulative release of fluoride from the three tested varnishes differed significantly in each hour of observation and at each acidity of the
environment ($p < 0.001$) (Table 1). For $pH = 7$ at 168 h, a significant difference occurred only between Embrace Varnish and MI Varnish ($4.82$ vs. $6.47$ mg; $p = 0.012$).

Table 1. Cumulative release of fluoride from three varnishes during subsequent observation hours and results of variance analysis (ANOVA) and post hoc tests. Descriptive data were presented as mean ± SD (± standard deviation).

<table>
<thead>
<tr>
<th>pH</th>
<th>Time/Duration (h)</th>
<th>DP N = 10</th>
<th>EV N = 10</th>
<th>MV N = 10</th>
<th>ANOVA $p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1</td>
<td>$1.47 ± 0.27$ $^{1,2}$</td>
<td>$2.34 ± 0.50$ $^{1,3}$</td>
<td>$3.68 ± 0.21$ $^{2,3}$</td>
<td>&lt;0.001</td>
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<td>2</td>
<td>$3.67 ± 1.02$ $^{1,2}$</td>
<td>$4.18 ± 0.78$ $^{1,3}$</td>
<td>$6.52 ± 0.79$ $^{2,3}$</td>
<td>&lt;0.001</td>
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<tr>
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<td>24</td>
<td>$6.85 ± 0.96$ $^{1,2}$</td>
<td>$6.01 ± 1.11$ $^{1,3}$</td>
<td>$10.63 ± 0.77$ $^{2,3}$</td>
<td>&lt;0.001</td>
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<td>48</td>
<td>$9.24 ± 1.11$ $^{1,2}$</td>
<td>$7.02 ± 1.15$ $^{1,3}$</td>
<td>$11.23 ± 0.80$ $^{2,3}$</td>
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<td>168</td>
<td>$9.86 ± 1.10$ $^{1,2}$</td>
<td>$7.51 ± 1.17$ $^{1,3}$</td>
<td>$11.52 ± 0.84$ $^{2,3}$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>$1.19 ± 0.30$ $^{1,2}$</td>
<td>$2.43 ± 0.51$ $^{1,3}$</td>
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<tr>
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<td>$3.30 ± 0.81$ $^{1,2}$</td>
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<td>$8.80 ± 2.48$ $^{1,2}$</td>
<td>$6.45 ± 1.08$ $^{1,3}$</td>
<td>$9.00 ± 0.75$ $^{2,3}$</td>
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<td>168</td>
<td>$9.30 ± 2.54$ $^{1,2}$</td>
<td>$6.83 ± 1.10$ $^{1,3}$</td>
<td>$9.33 ± 0.77$ $^{2,3}$</td>
<td>0.003</td>
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<tr>
<td>7</td>
<td>1</td>
<td>$1.16 ± 0.53$ $^{1,2}$</td>
<td>$2.41 ± 0.77$ $^{1,3}$</td>
<td>$1.64 ± 0.20$ $^{2,3}$</td>
<td>&lt;0.001</td>
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<td>$2.14 ± 0.89$ $^{1,2}$</td>
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<td>$3.13 ± 0.35$ $^{2,3}$</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>24</td>
<td>$3.87 ± 1.63$ $^{1,2}$</td>
<td>$4.29 ± 0.80$ $^{1,3}$</td>
<td>$5.69 ± 0.47$ $^{2,3}$</td>
<td>&lt;0.001</td>
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<tr>
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<td>48</td>
<td>$5.05 ± 1.69$ $^{1,2}$</td>
<td>$4.64 ± 0.82$ $^{1,3}$</td>
<td>$6.31 ± 0.43$ $^{2,3}$</td>
<td>0.007</td>
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<tr>
<td></td>
<td>168</td>
<td>$5.72 ± 1.82$ $^{1,2}$</td>
<td>$4.82 ± 0.85$ $^{1,3}$</td>
<td>$6.47 ± 0.43$ $^{2,3}$</td>
<td>0.016</td>
</tr>
</tbody>
</table>

DP—Duraphat, EV—Embrace Varnish, MV—MI Varnish, $^1$ DP vs EV, $p < 0.05$; $^2$ DP vs MV, $p < 0.05$; $^3$ EV vs MV, $p < 0.05$.

In the investigated groups, fluoride was released throughout the experiment, with the rate of release decreasing over time for all tested products, with a view to the differences between the first-generation (Duraphat) and second-generation (Embrace Varnish and MI Varnish). The hourly drop in fluoride release from three types of varnish in different pH environments is shown in Figure 2. The analysis uses mathematical models of fluoride release. Due to the non-linear nature of the obtained fluoride release rate scatterplots as a function of time, the results of the measurements were approximated by the exponential function. For empirical data obtained for each of the three types of varnish and three levels of pH, the following types of exponential models were matched:

$$y = b_0 \times \exp(b_1 \times x)$$

where $y = F$ (ppm/h)—hourly fluoride release and $x = t$ (h)—fluoride release duration. The parameters of the $b_0$ and $b_1$ models were estimated using the quasi-Newton method.
Based on the obtained data for all tested varnishes in all three environments (pH = 4, 5 and 7), in the first hours, the fluoride release rate is highest, and in the hours that follow, it rapidly decreases. For MI Varnish and Embrace Varnish, the fluoride release proceeds in a similar way and is completed after about 24 h, while for Duraphat, it takes much longer (at least 48 h).

**Figure 2.** The course of the hourly release of fluoride in three types of varnish in three different pH environments.

The absorption measurements (see Figure 3) of all three varnishes reveals that each of them has more than 98% of light transmission in the visible and NIR regions. Differences are visible in the UV region where MI Varnish is almost transparent (above 85% of light transmission), while transmission of MI Varnish and Embrace Varnish drops to about 60% at 275 nm.

**Figure 3.** Transmittance spectra of Duraphat, Embrace Varnish and MI Varnish in UV-VIS-NIR region.
3. Discussion

Application of fluoride varnish involves the use of high concentrations of fluoride salts suspended in resin, which allows them to remain on the surface of the teeth for a period of time. The saliva-dissolved salts release fluoride ions, thus causing accumulation in higher concentrations (above 100 ppm) both on the enamel surface and in the enamel itself, as well as in the plaque. The presence of fluoride attracts calcium and phosphate ions from saliva or dissolved hydroxyapatites, forming small grains of calcium fluoride with integrated phosphates [3,10,11,24–26,28,31–33]. Since pure CaF$_2$ crystal is more cubic than spherical, the resulting spherical deposits are described as calcium fluoride-like compounds, which, on the one hand, block the diffusion of acids, while on the other hand form a fluoride reservoir that is stable and insoluble in a pH-neutral environment. Phosphate ions increase its solubility compared with pure CaF$_2$ [5,34–36]. In the event of a decrease in pH, the membrane coating is dissolved and fluoride ions are released and embedded in the enamel structure. A decrease in fluoride concentration below 100 ppm and an increase in pH result in saturation of the enamel in relation to the surrounding fluid and inhibition of the process described above [1,3,5,7,10,24,26,33,37,38]. According to many researchers [27,33,35,39–43], the CaF$_2$ formation process, in addition to a sufficiently high concentration of fluoride ions, is also affected by calcium and phosphate ions present in the oral environment, and the quantity of which is insufficient to bind the whole released fluoride [33,44–47]. The supply of these ions in second-generation varnishes, according to Attin et al. [41], causes deposits of more calcium fluoride on the enamel surface, which in turn enhances the therapeutic effect [32,44,45,48–59]. According to Ogaard [35], these deposits can be observed in vitro at a high magnification up to 4 months after application. In vivo, due to chewing, swallowing, speaking and individual hygienic procedures, this time is much shorter and varies from several hours, according to Beltran et al. [5]; to 24 h, according to Schemehorn et al. [59] and Fernández et al. [60]; or up to 3 days, according to Hatrick et al. [61]. It follows that in the oral environment, the release of ions from fluoride varnishes should take place in the shortest possible time before they are completely lost [45].

All three varnishes used in the study had the same fluoride ion content (2.26% F$^-$ in 5% NaF). In order to minimize the differences between specimens, uniform amounts of varnish were applied to the normalized area of exposed enamel, where possible—and quickly, so as to reduce the possible change in the weight of the varnish due to evaporation of volatile components. Duraphat, considered by many [61] as a so-called gold standard, is classified as the first-generation varnish, in which 5% NaF is the active ingredient. In addition to 5% NaF, the second-generation varnishes used in this experiment contain CPP-ACP (MI Varnish) and calcium phosphate coated with xylitol (CXP) (Embrace Varnish). Many researchers [25,27,44–46,55,57,62,63] claim that caseine phosphopeptide complex with amorphous calcium phosphate is the best technology, enabling both the stable combination of high concentrations of calcium, phosphate and fluoride ions with the tooth surface and plaque, as well as high bioavailability and the possibility of free movement into the enamel, resulting in active remineralization of subsurface lesions. According to Shen et al. [22], Cochrane et al. [44] and Sleibi et al. [64], it also causes faster and easier release of fluoride ions in the first 24 h after application. In turn, according to Yapp et al. [65], it is better to place calcium and phosphate ions, but in the form of CXP, into permeable resin, as is the case with Embrace Varnish, preventing uncontrolled separation of fluoride salts from the resin matrix and allowing uniform and long-term dosing. Xylitol, additionally, provides a longer antibacterial effect [25,66].

The authors of this paper hypothesized that there would be no differences in the release of fluoride ions associated with the type of varnish applied, the time that elapsed from the application, and the pH of the artificial saliva. The results obtained proved to be highly significant for all three aspects ($p < 0.001$), which resulted in the rejection of the zero hypothesis. In the experiment, all varnishes released fluoride throughout the test period and showed a gradual decrease in time, while the shape and slope of the release curves were different, as were the values of cumulative release of fluoride and
the maximum fluoride levels, which is consistent with previous studies [43,64,67–69]. In any case, regardless of the pH of the environment and the type of varnish, the release of fluoride was at the highest level during the first two hours after application, as shown in the studies carried out by Sleibi et al. [64], Carvalho et al. [26], Shen et al. [70], Jablonowski et al. [43], Cochrane et al. [45] and Milburn et al. [27]. In the case of second-generation varnishes, fluoride was released in two phases, described earlier by Pichiaaukrit et al. [71]. The first phase is the so-called phase of initial rapid release of large amounts of fluoride, which took place in the first two hours after application, reaching the second phase—referred to in the literature as the so-called “plateau”—after 24 h, characterized by a much slower, constant increase of fluoride. Similar results were obtained by other researchers, yet Milburn et al. [27] observed a dynamic increase in fluoride released from MI Varnish during the first four hours, rather than two hours, and a ‘plateau’ between 8 and 12 h after application, while Comar et al. [40] observed these patterns after three hours and 12 h, respectively. As for the first-generation varnish, Duraphat, the increase in the amount of fluoride released was slower but systematic with a slow decrease after 48 h without the “plateau” phase, which is consistent with the observations by Castillo et al. [72] and Fernández et al. [60]. After 24 h, both second-generation varnishes released 90% of the total amount of fluoride measured on average, which is consistent with the study by Shen et al. [22], where, within 24 h, MI Varnish released 96% of the total fluoride. Based on previous considerations, which assumed that the time to release fluoride ions in vivo should not exceed 24 h, such a feature of the varnish profile seems to be very desirable. In addition, according to previous observations by Clifton et al. [10], a sufficiently high initial concentration of fluoride enables the formation of more calcium fluoride on the surface of the enamel and facilitates its binding to the tissues of the tooth. In the study, the cumulative release of fluoride was at the highest level for MI Varnish regardless of the pH of the environment, which seems consistent with the research conducted by other authors. Similar results were obtained by Cochrane et al. [45], where MI Varnish ranked ahead of Clinpro White, Enamel Pro, Bifluoride 5 and Duraphat. This confirms the thesis that the addition of calcium and phosphate ions in the form of CPP-ACP complex supports the release of fluoride ions.

This study shows that similar products (varnish vs. varnish) with identical fluoride concentrations, identically applied on surfaces prepared in the same manner, can present different parameters of fluoride release, as previously noted by other researchers [22,27,43,67,70,72,73]. The resulting variability in the fluoride release models may be related to the composition of the varnishes, i.e., the type of resin used as a matrix for the components contained in it, the presence of additional substances, and the consistency, homogeneity and viscosity of the varnish or the particle size [22,35,43,45,64,67,70,72,73]. The significantly higher cumulative fluoride release at each measuring point in MI Varnish may be due to several factors. Cochrane et al. [17], Schemehorn et al. [59] and Cross et al. [74] emphasize very good solubility of the CPP-ACP complex itself in aqueous solutions, which can directly accelerate the release of fluoride. Some researchers pointed to the behavior of polyvinyl acetate polymer, i.e., the so-called PVA, present in MI Varnish. In the study, it was noted that it also showed great ease and speed of dissolution in water [75], and at the same time, the maximum release took place within the first hour after the application [76]. In addition, its solubility increased in the presence of organic solvents (in this case, ethanol), which facilitated its rapid evaporation, thus leaving free spaces for easier and faster contact with water [76]. In addition, based on recent reports, the constitution of CPP-ACP varnish molecules—which are smaller and more homogeneous than those of other varnishes—can cause their faster dissolution and easier ion release [64].

The pH of the environment turned out to be an additional factor having a highly significant ($p < 0.001$) effect on the rate of fluoride ion release. The greatest cumulative release of fluoride was achieved at pH = 4 for all tested varnishes. Slightly lower amounts were measured for pH = 5, but when comparing acidic environments, these differences were not statistically significant. The level of released fluoride in the neutral environment
was significantly lower than in environments with pH = 4 and pH = 5, as confirmed by post hoc tests. In addition, between the 48th and 168th hour, the decrease in fluoride release in the environment with pH = 4 was greater than in the environment with pH = 5, while the decrease in fluoride release in the environment with pH = 7 was insignificant. In the literature—although there is relatively little research comparing the fluoride release depending on the pH of the environment—attention is paid to the effect of low pH on both the release of fluoride ions and on the formation of calcium fluoride [3,24]. Ten Cate et al. [24], when comparing the formation of calcium fluoride after application of 2% neutral NaF with acidified fluoride preparations, found the presence of significantly larger amounts of CaF$_2$ deposited on the surface of the enamel. Interesting conclusions were also drawn by Twetman et al. [77], who showed that Fluor Protector, containing the lowest concentration of fluoride ions in relation to other varnishes but low in pH, initiates the formation of the largest amount of CaF$_2$ on the surface of the enamel. This was explained by the affinity of the fluoride released in the acidic environment to the enamel that was higher than in neutral conditions. This was confirmed by Mohd Said et al. [38], who showed that in an acidic environment, the concentration of calcium ions and phosphates in carious lesions is higher than in the fluids surrounding the tooth, which promotes increased absorption of fluoride by the tooth structure. However, it should be emphasized that the behaviour of different varnishes in an acidic environment is not the same, and that it would be wrong to assume that it always causes faster release of ions. This was demonstrated by Lippert [78] and Shen et al. [22] in their research, where only a portion of fluoride-containing preparations released more fluoride at low pH. As for MI Varnish, the pH value an additional factor influencing the balance between free and loosely bound calcium, phosphorus and fluoride ions, affecting their bioavailability, and, according to Cochrane et al. [17] and Cochrane et al. [44], its decrease should promote the release of ions from the CPP complex associated with plaque.

However, when analyzing the obtained results, it should be always noted that in vitro models are in some way limited by inadequate selection of biological conditions and artefacts associated, for example, with reaction conditions, and even the most accurate simulation of the in vitro oral environment does not fully mimic in vivo conditions. This should always be taken into account when in vitro data are transferred to in vivo conditions.

4. Conclusions

The study showed that in vitro fluoride release is affected by the type of varnish, the time elapsed from application and the pH of the environment, and their interdependencies seem to be of high statistical significance. All the varnishes investigated—Duraphat, Embrace Varnish and MI Varnish—regardless of the pH of the environment, released fluoride during 168 h of testing, yet the most dynamic part of the process took place in the first two hours following application. For second-generation varnishes, the release took place in two phases, with a clear marking of the second phase of the so-called plateau. In the acidic environment, the release of fluoride was significantly higher than in the neutral environment for all investigated varnishes, with no change in the overall fluoride release profile, with maximum fluoride release in the first two hours after application. All the varnishes investigated have more than 98% of light transmission in the visible region, which makes them invisible on teeth.

5. Materials and Methods

In vitro studies were conducted on 45 human teeth (premolars and molars) that were free from caries, enamel defects and demineralization, removed for orthodontic reasons after obtaining the prior consent of the patients. Each tooth was cut along the long axis with the buccal and palatal surface intact. Until the start of the study, the specimens were stored in thymol solution. Each specimen, after washing and drying, was coated with acid-resistant varnish while leaving exposed enamel (4 mm/4 mm) on the smooth buccal and palatal surface (Figure 4a,b).
Three fluoride varnishes were used for the purposes of the study: one first-generation varnish (Duraphat) and two second-generation varnishes (MI Varnish and Enamel Varnish). Table 2 provides information on the type and composition of the applied agents.

Table 2. Type and composition of studied varnishes.

<table>
<thead>
<tr>
<th>Fluoride Varnish</th>
<th>Code</th>
<th>Manufacturers</th>
<th>Ingredients</th>
</tr>
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<tbody>
<tr>
<td>Colgate® Duraphat Varnish</td>
<td>DV</td>
<td>Colgate Oral Care, Sydney, NSW, Australia</td>
<td>30–60% colophonium, 10–30% ethanol, 5% sodium fluoride, other ingredients</td>
</tr>
<tr>
<td>MI Varnish™</td>
<td>MV</td>
<td>GC, Tokyo, Japan</td>
<td>30–50% polyvinylacetate, 10–30% hydrogenated rosin, 20–30% ethanol, 5% sodium fluoride, 1–5% CPP-ACP, 1–5% silicon dioxide</td>
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<tr>
<td>Embrace Varnish™</td>
<td>EV</td>
<td>Pulpdent, Watertown, MA, USA</td>
<td>5% natrium fluoride with CXP (xylitol coated calcium and phosphate)</td>
</tr>
</tbody>
</table>

The test material was divided into nine groups of 10 specimens each, depending on the type of varnish applied and the pH of the environment (Table 3).

Table 3. Experimental groups.

<table>
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<tr>
<th>Fluoride Varnish</th>
<th>Group</th>
<th>pH of Artificial Saliva</th>
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<tr>
<td>Duraphat</td>
<td>DV/A</td>
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<tr>
<td></td>
<td>DV/B</td>
<td>5.0</td>
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<tr>
<td></td>
<td>DV/C</td>
<td>7.0</td>
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<tr>
<td>MI Varnish</td>
<td>MV/A</td>
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<td>MV/B</td>
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<tr>
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<td>MV/C</td>
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<tr>
<td>Embrace Varnish</td>
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</tbody>
</table>

The prepared specimens were weighed before and after the application in order to accurately determine the amount of the preparation applied, using a Radwag laboratory balances with a readability of 3 decimal places. The specimens were then placed in sealed plastic containers and immersed in 5 mL of artificial saliva at pH = 4.0 for groups DV/A, MV/A and EV/A; pH = 5.0 for groups DV/B, MV/B and EV/B; and pH = 7.0 for groups DV/C, MV/C and EV/C. The composition of artificial saliva was as follows: NaCl (0.4 g), KCl (4.0 g), urea (1.0 g), Na₂S·9H₂O (0.005 g), NaH₂PO₄·2H₂O and CaCl₂·2H₂O (0.795 g). pH = 7.0 was achieved with 1 M NaOH, whereas pH = 4.0 and 5.0 were achieved with the use of 1 M HCl. During the measurements, the specimens were stored at 37 °C. The...
determination of fluoride levels in the artificial saliva environment was performed three times each after 1, 2, 24, 48 and 168 h, and followed by calculation of the average value. In any case, after the measurement period, 5 mL of eluate was used for the determination; then the sample was dried and placed into a fresh artificial saliva solution. The ORION 9609 Model ion selective electrode combined with the CPI-551 Elmetron (Thermo Fisher Scientific Co., Waltham, MA, USA) microcomputer were used for the determination of fluoride ions. The system was calibrated before each subsequent determination using TISAB. Measurements of the released fluoride ion concentration were expressed in ppm, later converted into μmol/L and in relation to the quantity of applied varnish converted into μg/mg. In the study, the cumulative level of ion release and the increase in release measured within specified time intervals and for a unit of time, namely, 1 h.

The statistical analysis was carried out using the STATISTICA v. 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). The consistency of distribution for measured quantitative parameters with normal distribution was verified with the Shapiro–Wilk test. The critical significance level was \( p < 0.05 \). When comparing the mean values of the analyzed parameters in subgroups, one-factor analysis of variance (ANOVA) was used. For multiple comparisons (post hoc tests), Tukey’s least significant difference (LSD) test was used.

The zero hypothesis assumed that there would be no differences in the fluoride ion release associated with the time that passed from application, the type of varnish used and the pH of the artificial saliva.

The room temperature electron absorption spectra were measured in the 275–750 nm spectral range using a Cary-Varian 5E UV–vis-NIR spectrophotometer (Varian Medical Systems, Palo Alto, CA, USA) [7].

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