The aim of this in vitro study was to determine the fluoride concentration in silver diamine fluoride (SDF) products and their bioavailability with demineralized dentine. The products evaluated (expected fluoride concentrations) were: I: Saforide 38% (45,283 ppm F); II: Advantage Arrest 38.3 to 43.2% (45,283 to 51,013 ppm F); III: Ancárie 12% (14,100 ppm F); IV: Ancárie 30% (35,400 ppm F), V: Cariestop 12% (14,100 ppm F) and VI: Cariestop 30% (35,400 ppm F). The fluoride concentration was evaluated using an ion-specific electrode (ISE) by direct technique, which was confirmed after microdiffusion. The pH of the products was determined with a pH test strip. For the bioavailability test, demineralized dentine slabs were treated with one of the products for 1 min. Loosely (CaF$_2$-like) and firmly-bound fluoride (FAp) were determined. The fluoride concentration found in the products (mean±SD; ppm F) by the ISE direct technique was: I:53,491±554; II:57,249±1,851; III:4,814±268; IV:5,726±43; V:10,145±468; VI:11,858±575; these values were confirmed after microdiffusion (t-test; p>0.05) and disagree with the declared by the manufacturers. The pH of Ancárie 12 and 30% was 6.0 and 4.5, respectively, in disagreement with the alkaline pH expected for SDF solution and found in the other products evaluated. There was no correlation between either CaF$_2$-like (r=0.221; p=0.337) or FAp (r=-0.144; p=0.830) formed in demineralized dentine and fluoride concentration found in the products. The problems of pH and fluoride concentration found in available professional commercial SDF products suggest that they are not under sanitary surveillance.

**Fluoride Concentration in SDF Commercial Products and Their Bioavailability with Demineralized Dentine**

Aline Laignier Soares-Yoshikawa, Jaime Aparecido Cury, Cíntia Pereira Machado Tabchoury

1Department of Biosciences, Piracicaba Dental School, UNICAMP – Universidade Estadual de Campinas, Piracicaba, SP, Brazil

Correspondence: Prof. Jaime Aparecido Cury, Av. Limeira, 901, 13414-903 Piracicaba, SP, Brasil. Tel: +55-19-2106-5303. e-mail: jcury@unicamp.br

**Key Words:** fluorides, dental caries, cariostatic agents, dentine, demineralization.

**Introduction**

Since untreated dental caries of permanent and primary teeth affect billions of people around the world (1), silver diamine fluoride (SDF) seems to be a possible and feasible alternative to treat this condition. It has been used to arrest (2) and prevent new caries lesions in primary teeth (3) and in dentine (4) and to control root caries in elderly people (5). Although clinical trials have shown that the effect is concentration-dependent (6–8), SDF products with different concentrations (12, 30 and 38%) are available in the market. In addition, based on scientific evidence, only one product (Saforide, 38% SDF) has been considered effective to clinically control caries (5,8,9). Therefore, before being launched in the market, commercial SDF products should be submitted to a minimum test to evaluate their anti-caries potential.

SDF is an alkaline and colorless solution, that contains fluoride and silver ions in a complex with ammonia [AgF(NH$_3$)$_2$] (10). It was originally formulated in Japan at 38% of concentration (w/v) and named Saforide. If Saforide were produced from the salt AgF(NH$_3$)$_2$, it would be expected in the product 44,800 ppm F (µg/mL) and 253,870 ppm of silver in an alkaline pH. However, AgF(NH$_3$)$_2$ does not exist as a solid pure salt to prepare a solution. The SDF solution can be prepared, for example, from Ag$_2$O, that is produced from the reaction between sodium hydroxide (NaOH) with silver nitrate (AgNO$_3$). Then, the Ag$_2$O solid is filtered and fluoridic acid (HF) is added first, followed by ammonia (NH$_3$) addition. Therefore, the preparation of SDF solution is not trivial and might have an impact on the quality of the commercial product launched in the market. Recently, a 38% SDF product equivalent to Saforide, named Advantage Arrest, was launched in the American market and SDF products with 12 and 30% can be found in Brazil (10,11). Therefore, the manufacturers of SDF should show results of quality control tests that their products were subjected to before making them available in the market.

Indeed, any dental professional product should be evaluated before clinical use. A basic laboratorial test should check if the active agents in the product are chemically available and show bioavailability. Considering that SDF products contain silver and fluoride as active agents, but the mechanism of action of silver is not completely understood (12), the anti-caries potential of SDF could be evaluated by the quality of fluoride present in the product as the mechanism of action of high concentrated...
professional fluoride products is very well known (13). Thus, fluoride should be available (soluble) and in the required concentration in SDF products to present the anti-caries effect. Also, considering that SDF efficacy is to arrest caries progression in dentine, SDF products should show that they present fluoride bioavailable to react with carious dentine (bioavailability with demineralized dentine) in an alkaline pH.

Some commercial SDF products have been evaluated in terms of fluoride availability, but not bioavailability. Conflicting results have been found; lower or higher concentrations than those declared by the manufacturer have been found (14,15). The fluoride concentration in these products was evaluated using the ion-specific electrode (ISE) by the direct technique. Distinct fluoride concentration found than the expected could be explained by the fact that SDF commercial products are not prepared from pure AgF(NH₃)₂ salt. In fact, the manufacturer of Advantage Arrest admits that its product contains from 45,283 to 51,013 ppm F (38.3 to 43.2%, w/v, of SDF). However, lower F concentration than the expected found in the analysis made with F-ISE by the direct technique (14,15) can be due to interferents (16). Therefore, the concentration determined with F-ISE should be confirmed after fluoride microdiffusion (16), but this was not done by Mei et al. (14) and Crystal et al. (15).

In addition, laboratorial evaluation of SDF commercial products in terms of fluoride bioavailability has not been conducted. SDF at 38% of concentration presents 44.800 ppm F, a very high fluoride concentration to form CaF₂-like products in demineralized dentine to interfere with caries progression (17). However, SDF products must have alkaline pH (around 10) to stabilize the product (9) and it is well known that the reactivity of fluoride with enamel-dentine (bioavailability) is greater at low pH (18). Thus, in SDF products, the low fluoride bioavailability due to the alkaline pH may be compensated by the high fluoride concentration. Therefore, the laboratorial determination of the pH of commercial products is very important either to check if it was properly manufactured or to interpret any misleading results of bioavailability.

Thus, the present study aimed to evaluate the anti-caries potential of SDF commercial products in terms of fluoride availability and bioavailability. Fluoride availability was determined with F-ISE by the direct technique and checked after microdiffusion. Bioavailability was determined by the concentrations of loosely (CaF₂-like) and firmly-bound fluoride (FAp) formed in demineralized dentine.

**Material and Methods**

**Experimental Design**

Six SDF commercial products were evaluated (Table 1).

<table>
<thead>
<tr>
<th>Products Name</th>
<th>Manufacturer</th>
<th>Composition shown in the label</th>
<th>% of SDF and ppm F declared or expected*</th>
<th>pH</th>
<th>Lots</th>
<th>Manufacturing date</th>
<th>Expiration date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saforide</td>
<td>Toyo Seiyaku Kasei, Osaka, Osaka, Japan</td>
<td>Silver Diamine Fluoride</td>
<td>38% - [45,283 ppm F]*</td>
<td>Not mentioned</td>
<td>609 RA 704 RA</td>
<td>Not mentioned</td>
<td>Sep 2019 Apr 2020</td>
</tr>
<tr>
<td>Advantage Arrest</td>
<td>Elevate Oral Care, West Palm Beach, FL, USA</td>
<td>Silver Diamine Fluoride</td>
<td>38.3 to 43.2% w/v, (45,283 to 51,013 ppm F)</td>
<td>Not mentioned</td>
<td>16092 16175</td>
<td>Not mentioned</td>
<td>Jul 2019 Nov 2019</td>
</tr>
<tr>
<td>Ancárie 12%</td>
<td>Maquira, Maringá, PR, Brazil</td>
<td>Fluoridic acid, silver nitrate, ammonia hydroxide, deionised water</td>
<td>12% [14,100 ppm F]*</td>
<td>8.5-9</td>
<td>172917 354217 861016</td>
<td>Apr 2017 Jul 2016 Jan 2018</td>
<td>Apr 2019 Jul 2019 Jan 2018</td>
</tr>
<tr>
<td>Ancárie 30%</td>
<td>Maquira, Maringá, PR, Brazil</td>
<td>Fluoridic acid, silver nitrate, ammonia hydroxide, deionised water</td>
<td>30% [35,400 ppm F]*</td>
<td>8.5-9</td>
<td>639515 002316 173117</td>
<td>May 2015 May 2016 Apr 2017</td>
<td>May 2017 Jun 2018 Apr 2019</td>
</tr>
<tr>
<td>Cariestop 12%</td>
<td>Biodinâmica, Ibiaporã, PR, Brazil</td>
<td>Fluoridic acid, silver nitrate, ammonia hydroxide, deionised water</td>
<td>12% [14,100 ppm F]*</td>
<td>8.5</td>
<td>917/15 310/16 167/17</td>
<td>Not mentioned</td>
<td>Oct 2018 May 2019 Mar 2020</td>
</tr>
<tr>
<td>Cariestop 30%</td>
<td>Biodinâmica, Ibiaporã, PR, Brazil</td>
<td>Fluoridic acid, silver nitrate, ammonia hydroxide, deionised water</td>
<td>30% [35,400 ppm F]*</td>
<td>8.5</td>
<td>767/14 927/14 450/15</td>
<td>Not mentioned</td>
<td>Aug 2017 Sep 2017 May 2018</td>
</tr>
</tbody>
</table>

*Expected according to the % of SDF declared by the manufacturer.
All analyses were carried out within the expiration date and as soon as the products were purchased. For the fluoride determination, the products were diluted and aliquots were analyzed using ion-specific electrode (ISE) by the direct technique, whose reliability was checked after microdiffusion. The pH of the products was also determined. For the bioavailability, root dentine slabs from bovine incisor were used. Caries-lesions were induced in the slabs, which were sectioned in two halves (Fig. 1) for a paired experimental design. One half was used as a control for fluoride concentration (baseline data) and the other was treated with the respective product for 1 min (n=10/group). Loosely (CaF₂-like) and firmly-bound fluoride (FAp) were determined in both hemi-slabs. The results found in the baseline hemi-slabs were subtracted from the treated hemi-slabs and expressed as µg F/cm². The fluoride concentrations in the products found by direct technique and after microdiffusion were compared by t-test (α=0.05). CaF₂-like and FAp data were analyzed by one-way ANOVA followed by Tukey’s test (α=0.05).

**Determination of Fluoride Concentration and pH in the SDF Products**

Different lots of the products described in Table 1 were purchased and each product was diluted by volume with purified water according to fluoride concentration found in a pilot study. Thus, Saforide and Advantage Arrest were diluted 5,000 times; Ancaríe 12 and 30% - 500 times and Cariestop 12 and 30% - 1,000 times. From each bottle, the dilutions were made in triplicates. From the same dilution, aliquots were used for fluoride determination by both techniques: direct and after microdiffusion. Fluoride was determined using a F-ISE (Orion model 96-09, Orion Research Incorporated, Cambridge, MA, USA) coupled to an ion analyzer (Orion Star A214, Thermo Fisher Scientific Inc., Waltham, MA, USA). For the analysis by the direct technique, the apparatus was previously calibrated with standards ranging from 2 to 32 µg F/mL, prepared in TISAB II 50% (v/v). For the analysis after microdiffusion (16), the electrode was previously calibrated with fluoride standards solutions, ranging from 2 to 64 µg F/mL, diffused as the samples. The results were expressed in ppm (µg F/mL). The average coefficient of variation (CV) of the triplicates was 1.8% for direct technique and 2.8% for microdiffusion technique. The accuracy of both fluoride determinations was validated using reference standards fluoride (Orion 940907, Thermo Fisher Scientific Inc.) and the average coefficient of variation from the triplicates was 1.7% for the direct technique and 2.3% for analysis after microdiffusion. For the pH determination, a pH test strip (Merck KGaA, Darmstadt, Germany; lot number: HC386285) was used (±0.5 units of pH). The strip was immersed directly in the solution of each bottle and kept in contact with the product for 5 s. Then the pH test strip was visually compared to the chart provided with the strips.

**Dentine Slabs Preparation**

Bovine incisor teeth were stored in 2% formaldehyde solution for a period of at least 30 days (19). The incisor teeth were sectioned with a low-speed diamond blade, separating crowns and roots. Then, dentine slabs (7x4x2 mm) were obtained from the roots, polished using aluminum oxide abrasive papers (#600, #800 and #1200), fixed on acrylic support with wax (Fig. 1) and stored in relative
humidity. The slabs were selected according to their baseline surface hardness (SH), which was determined by making 3 indentations with 5-g load for 5 s, 100 µm apart, using a Knoop hardness tester coupled to FM-ARS 900 software (Future Tech Corp., Kawasaki, Japan). Before the SH determination, dentine slabs were allowed to dry for at least 30 min to standardize the measurements (20). The selection criteria for the sound dentine slabs were based on mean and standard deviation of SH of each slab. The slabs with intra-variability higher than 10% of their average individual hardness and those with inter-variability higher than 15% of the average hardness calculated for all slabs initially obtained (variability between slabs) were excluded. Thus, the mean baseline SH of the selected sound dentine slabs was 40.1±6.7 kg/mm². Sound dentine slabs were then immersed in artificial saliva for 24 h (1.5 mM Ca, 0.9 mM P, 150 mM KCl and 0.1 M Tris buffer, pH 7; 2 mL/mm²) to allow root dentine mineral gain and to minimize further ionic changes (21). Afterwards, for demineralization, the slabs were immersed in 0.05 M acetate buffer, pH 5.0, containing 1.4 mM Ca, 0.91 mM Pi and 0.06 µg F/mL for 16 h at 37°C (22). Then, SH analysis was repeated and the percentage of SH loss (%SHL) was subsequently calculated. The selection criteria for the demineralized dentine slabs were based on mean and standard deviation of %SHL of all slabs. The slabs with inter-variability higher than 20% of the average %SHL calculated were excluded. The mean %SHL of the selected dentine carious slabs was 65.4±2.3.

Each selected deminerlized dentine slab was sectioned in two halves (3.5x4 mm). Only the outer dentine surface was exposed to the treatments and the other surfaces were isolated with wax. One half was used as a control to measure the fluoride concentration (baseline data) and the other was treated with the respective product (Fig. 1).

Treatment of Slabs

For the analysis of bioavailability, the demineralized hemi-slabs were randomized to the treatments (n=10/group). The treatments were applied with microbrush (only to spread the product) and, after 1 min, the slabs were individually washed with purified water from a wash bottle for 30 s.

Determination of Loosely (CaF₂-Like) and Firmly-Bound Fluoride (FAp)

For the determination of CaF₂-like products, dentine slabs were removed from the acrylic support and individually immersed in 0.5 mL of 1.0 M KOH for 24 h (23). Then, the extract was buffered with 0.5 mL of TISAB II containing 1.0 M HCl and TISAB II containing 1.0 M HCl 50% (v/v). For determination of firmly-bound fluoride (FAp), standards of fluoride solutions, in triplicate, ranging from 0.125 to 32 µg F/mL for determination of CaF₂-like products, prepared in 0.5 M KOH and TISAB II containing 1.0 M HCl 50% (v/v). For determination of firmly-bound fluoride (FAp), standards of fluoride solutions, in triplicate, ranging from 0.125 to 8 µg F/mL, were prepared in 0.25 M HCl and TISAB II containing 0.5 M NaOH 50% (v/v). The accuracy of the analysis was checked with a standard fluoride solution (Orion 940907, Thermo Fisher Scientific Inc.) and the average coefficient of variation from triplicates was 2.4%, for CaF₂-like analysis, and 2.9%, for FAp analysis.

The results found in the baseline hemi-slabs were subtracted from the treated ones. The amount of FAp found in the two layers of dentine removed were summed. The fluoride concentration was expressed as µg F/cm² for both analyses.

Table 2. Fluoride concentration found in the products* (µg F/mL; mean±SD), pH of the products and concentrations (µg F/cm²; mean±SD) of loosely (CaF₂-like) and firmly-bound fluoride (FAp) formed on demineralized dentine (n=10) according to the treatments with the SDF products evaluated

<table>
<thead>
<tr>
<th>Products/Treatments</th>
<th>µg F/mL (ppm F)*</th>
<th>pH</th>
<th>µg F/cm² CaF₂-like</th>
<th>µg F/cm² FAp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saforide 30%</td>
<td>53,490.9±554.1</td>
<td>10.0</td>
<td>75.9±25.4 A</td>
<td>2.1±1.1 A</td>
</tr>
<tr>
<td>Advantage Arrest 12%</td>
<td>57,249.4±1,851.3</td>
<td>10.0</td>
<td>89.9±34.1 A</td>
<td>1.8±1.0 A</td>
</tr>
<tr>
<td>Ancárie 12%</td>
<td>4,814.2±267.6</td>
<td>6.0</td>
<td>72.8±26.2 A</td>
<td>2.1±1.3 A</td>
</tr>
<tr>
<td>Ancárie 30%</td>
<td>5,726.2±42.89</td>
<td>4.5</td>
<td>98.0±28.5 A</td>
<td>3.0±1.1 A</td>
</tr>
<tr>
<td>Cariestop 12%</td>
<td>10,144.5±467.6</td>
<td>8.0</td>
<td>40.6±17.2 B</td>
<td>1.6±1.2 B</td>
</tr>
<tr>
<td>Cariestop 30%</td>
<td>11,858.4±575.4</td>
<td>8.0</td>
<td>82.9±23.1 A</td>
<td>1.2±0.6 B</td>
</tr>
</tbody>
</table>

*By the direct technique; no statistically significant difference (t-test; p>0.05) was observed in comparison with the analysis after microdiffusion. Treatments (data within columns) followed by distinct superscript letters statistically differ by Tukey test (p<0.01).
hemi-slabs and expressed in µg F/cm². The CaF₂-like and FAp data were analyzed using ANOVA one-way, followed by Tukey test (p<0.05). Correlation tests were conducted between fluoride concentration in the products and either the concentration of CaF₂-like or the concentration of FAp formed in dentine. For all analyses, the Statistical Package for the Social Sciences (SPSS, IBM, version 20.0; SPSS Inc., Chicago, IL, USA) was used and the significance level was set at 5%.

Results
The reliability of fluoride determination in the SDF products with F-ISE by the direct technique was proven, because they did not statistically differ (t-test; p>0.05) in comparison with the analysis made after microdiffusion (data not shown). Thus, table 2 shows the F concentration found in the SDF products by the direct technique. Saforide and Advantage Arrest presented higher F concentrations than expected (18% and 10% higher, respectively). For the other products, the fluoride concentration found was lower than expected (Ancárīe 12% was 66% lower, Ancárīe 30% was 84% lower, Cariestop 12% was 29% lower and Cariestop 30% was 66% lower).

Table 2 also shows that the SDF products Ancárīe 12 and 30% were formulated with acidic pH (around 6 and 4.5, respectively), but all the other products were alkaline and had a pH around 8 to 10. In relation to CaF₂-like formed on demineralized dentine, Table 2 shows that Ancárīe 30% presented greater bioavailability in comparison with Cariestop 12% (p<0.05), but it did not differ from the other SDF products evaluated (p>0.05). Regarding FAp formation, Ancárīe 30% was more reactive than Cariestop 12% and Cariestop 30% (p<0.05), but it did not differ from the other SDF products (p>0.05). In fact, there was no statistically significant correlation between the concentration of CaF₂-like (r=0.221; p=0.337) or FAp (r=−0.144; p=0.830) formed on demineralized dentine and the F concentration found in the products evaluated. The reason for these findings is discussed further.

Discussion
The preparation of SDF for professional fluoride application is a challenge when compared with fluoride gel, varnish or mousse products, because there is no AgF(NH₃)₂ salt to formulate the product, while F-gel, varnish and mouthrinse are prepared from commercial available NaF salt. Furthermore, if SDF products were properly prepared, they would have an alkaline pH.

The findings of the present study clearly show that the assertive above is valid and first the results of the pH determined in the products evaluated (Table 2) were discussed, considering its impact in the results of bioavailability found. Thus, although the manufacturer of Ancárīe has declared that its product should have alkaline, around pH 8.5-9.0, Ancárīe 12% and 30% presented a pH value of 6.0 and 4.5, respectively. These findings show that the manufacturer did not produce a solution to satisfy the stoichiometry of the complex AgF(NH₃)₂ expected in SDF products. Besides Ag⁺¹, F⁻¹ and NH₃, Ancárīe 12 and 30% contain NO₃⁻¹, as they were prepared by mixing “Fluoridic acid, silver nitrate and ammonia hydroxide” (Table 1). This unusual preparation of SDF is confirmed by a lower pH of Ancárīe 30% compared with Ancárīe 12% as more fluoridic acid was obviously used, but not necessarily neutralized with ammonia hydroxide. The other products analyzed presented alkaline pH (Table 2), but it is not a guarantee that they were formulated according to the formula AgF(NH₃)₂. Indeed, according to the manufacturer of Cariestop, its product is a simple mixture of “Fluoridic acid, silver nitrate and ammonia hydroxide in deionized water” (Table 1).

The results of fluoride concentration found in the products evaluated show (Table 2) that Saforide and Advantage Arrest were formulated close to the stoichiometry of AgF(NH₃)₂ complex expected, but the concentrations found were greater than the expected (around 20%). On the contrary, in the other SDF commercial products, fluoride concentrations were lower than those expected (28 to 84% lower) if the preparation had followed the chemical formula AgF(NH₃)₂. The difference between the values found and expected cannot be attributed to interferents in the analyses made with F-ISE, because the results found by the direct technique were confirmed after fluoride microdiffusion. The greater concentration in Advantage Arrest and the lower in Cariestop products agree with the findings of Crystal et al. (15) and Mei et al. (14), respectively. The current results found for Ancárīe and the confirmatory for Cariestop are alarming, suggesting that they are manufactured without a quality control. The necessity of sanitary surveillance of these products is evident when fluoride concentration of Ancárīe 12% vs. 30% or Cariestop 12 vs. 30% are compared (Table 2). In the products with SDF 30%, it would be expected 2.5 times more fluoride than 12%, but the difference was minimum. Considering that the products with 12 or 30% would have different clinical recommendations of use, the effect of 30% would not be better than 12%. Unfortunately, we were not able to determine Ag concentration in these products, but based on the present discussion we do not expect any difference from the findings of fluoride.

The results of pH and fluoride concentration in the SDF products evaluated had impact in the findings of bioavailability of fluoride with demineralized dentine. As the reactivity of fluoride with enamel-dentine is concentration dependent and the products evaluated...
differed around 12 times in fluoride concentration (from 4,800 to 57,000 ppm F), a correlation with CaF$_2$-like or FAp formed would be expected, but it was not found (Table 2). The explanation for the absence of correlation is due to the fact that the SDF products with lower fluoride concentration also present acid pH and the reactivity of fluoride is greater at low pH (18). Considering that SDF products must have alkaline pH (around 10) to stabilize the product (9), the results found are misleading because SDF products with acid pH might not have anticaries effect. Therefore, further studies should be conducted using biofilm models to check the stability of the products in terms of antimicrobial effects.

Nevertheless, the problems of manufacturing found for the SDF products Ancárie and Cariestop is an opportunity to discuss the clinical data of anticaries efficacy of these commercial products for professional fluoride application. Any product only should be clinically evaluated after being approved by preliminary laboratorial tests of quality control and anticaries potential. Thus, a systematic review (2) has concluded that 38% silver diamine fluoride (Saforide) is effective in arresting dentine caries, while conflicting results were found with products containing lower SDF concentration (Cariestop 12 and 30%). Considering that problems with Cariestop formulations have been reported since 2013 (14) and confirmed in the present study (Table 2), the conclusion that only SDF at 38% is effective to arrest dentine caries is not valid. In addition, another question is about ethics to conduct clinical trials with products presenting problems in the formulation, giving support to the conclusion of the present study.

In summary, the findings show that most SDF commercial products available are not being manufactured under a quality control system, requiring that they are subjected to sanitary surveillance to guarantee the anticaries effect promised.

Resumo

O objetivo deste estudo in vitro foi determinar a concentração de fluoreto nos produtos de diâmino fluoreto de prata (DFP) e sua biodisponibilidade com dentina desmineralizada. Os produtos avaliados (concentrações esperadas de fúloro) foram: I: Saforide 38% (45.283 ppm F); II: Advantage Arrest 38,3 a 43,2% (45.283 a 51.013 ppm F); III: Ancárie 12% (14.100 ppm F); IV: Ancárie 30% (35.400 ppm F); V: Cariestop 12% (14.100 ppm F) e VI: Cariestop 30% (35.400 ppm F). A concentração de fluoreto foi avaliada utilizando um eletrodo iônico específico (EIE) por técnica direta, que foi confirmada após microdifusão.

In summary, the findings show that most SDF commercial products available are not being manufactured under a quality control system, requiring that they are subjected to sanitary surveillance to guarantee the anticaries effect promised.

References


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