Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2025; 25:e240062
https://doi.org/10.1590/pboci.2025.093
ISSN 1519-0501 / eISSN 1983-4632

ORIGINAL ARTICLE

Effect of Different pH Media on Fluoride-releasing of Silver Diamine Fluoride in Primary Teeth: An in Vitro Study

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Academic Editor: Ana Maria Gondim Valença

Received: September 02, 2024 / **Review:** November 01, 2024 / **Accepted:** January 24, 2025

How to cite: Alboeid A, Alamri N, Al Gahtani M, Ibrahim MS, Khan AS, Gaffar BO. Effect of different pH media on fluoride-releasing of silver diamine fluoride in primary teeth: An in vitro study. Pesqui Bras Odontopediatria Clín Integr. 2025; 25:e240062. https://doi.org/10.1590/pboci.2025.093

ABSTRACT
Objective: To investigate the effect of different pH media on the concentration of fluoride-released ions from silver diamine fluoride (SDF) after its application on the enamel surface of primary molars. **Material and Methods:** A total of 33 specimens were prepared in sectioned parts of primary molar teeth with the following measurements (4×4 mm in height and width). Each specimen was immersed in an airtight polyethylene bottle containing 2 mL of pH media (neutral, alkaline, and acidic). The fluoride release pattern of each sample was taken on days 1, 2, 7, 15, and 30 using an Orion fluoride ion-selective electrode. **Results:** On day 1, acidic media showed the least mean fluoride release (8.83 ppm) compared to neutral (23.17 ppm) and alkaline media (18.92 ppm). There was a persistent decrease in the mean fluoride release at all time intervals for each media. On day 30, the fluoride release of acidic, neutral, and alkaline media was 0.477 ppm, 3.325 ppm, and 4.183 ppm, respectively. The acidic media showed a significant decrease in fluoride release ($p=0.001$) from day 1 to day 30 compared to neutral and alkaline media. However, non-significant differences were observed between neutral *vs.* alkaline media at all time intervals. **Conclusion:** The neutral group showed the highest fluoride release compared to other groups, where the different pH media strongly affect fluoride release from SDF material.

Keywords: Dental Caries; Tooth, Deciduous; Hydrogen-Ion Concentration.

Introduction

Silver nitrate was first introduced in Japan by Nishino, Yamaga, and others in the 19th century to arrest dental caries, followed by rapid development during the 20th century to create a more effective formulation [1]. The formulation started with Howe's ammoniacal silver nitrate, followed by silver fluoride and silver diamine fluoride (SDF) [2,3]. At the beginning of the 21st century, the SDF was used in China as a caries arresting agent for school children [4]. From 2005 to 2009 in Australia, a series of in vitro studies conducted by Knight et al. [5,6] and their group proved its effectiveness as a caries-arresting and antimicrobial agent. Later, it was used as a caries arresting agent in other parts of the world [7,8]. The topical application of SDF as a non-invasive dental treatment is currently gaining popularity worldwide [9].

The US Food and Drug Administration (FDA) approved the use of SDF as a desensitizing agent in 2014. In 2015, 38% SDF solution was the first commercial product available in the United States as a preventive and caries arresting agent [10]. In January 2016, a new Code on Dental Procedures and Nomenclature (CDT) D1354 allowed billing claims for off-label use of SDF as an interim caries arresting medicament [11,12]. A solution of 38% SDF ($Ag(NH_3)_2F$) is alkaline (pH=10) in nature, having a colorless solution containing 24-27% silver (Ag), 8.5-10.5% ammonia (NH3), and 5.0-6.0%fluoride (F) [13]. It is reported that the annual application of 38% SDF could arrest active root caries by 90% [14].

After three decades of infrequent studies, clinical trials with more rigorous experimental designs evaluated the effectiveness of the SDF in the management of dental caries [3]. A study focused on the incidence of new lesions reported that the prevented fractions were 77% and 73% after 2.5 years [15]. The effects of SDF are primarily the prevention of caries at the enamel and dentin levels since it contains fluoride (F), which mainly affects enamel, while the effect on dentin is predominantly by silver [2,16,17]. The literature reported that the application of SDF increased the bond strength of resin-based composite to carious-affected dentin [18]. SDF has been used as an inhibitor of caries in deciduous and permanent teeth because of the presence of silver compounds as a sterilizing agent. Moreover, it is used to promote the remineralization of enamel and dentin, reducing caries incidence because of its fluoride containment [10].

Studies were conducted to determine the SDF's initial contents, besides the short-term stability of fluoride and silver concentrations over 28 days, an inverse relationship was found between fluoride and silver contents by increasing fluoride ions and reducing silver ions in contrast [19,20]. Furthermore, a study that investigated the concentration of fluoride ions in saliva at three different intervals, which are before, immediately after, and one hour after 38% SDF application on enamel, concluded that fluoride concentration reached its peak immediately, and after one hour it had returned to the baseline [21]. However, the major limitation of these studies is that they were only experimented on human saliva, not on different pH media. The anti-cariogenic effect of fluoride-releasing materials depends on the amount and sustainability of fluoride release [22]. The pattern of release is typically characterized by an initial rapid release, followed by a significant reduction in the release rate after only a few days of immersion [23].

Several studies have been reported on the effect of pH on the fluoride release from various types of restorative materials [24-27]. However, the effect of pH on the release of fluoride from SDF has not been well documented. Considering the usefulness of SDF in caries prevention, the present laboratory study aimed to investigate the effect of different pH media on the concentration of fluoride-released ions from 38% silver diamine fluoride (SDF) after applying it to primary teeth at different time intervals. It was hypothesized that the concentration of fluoride released from SDF would be higher in an acidic pH medium.

Material and Methods

This was a randomized, controlled, single-blind (at the investigator level) laboratory study. Ethical permission was taken from the institutional review board (IRB-2021-02-483).

Teeth Selection and Sample Size

Using the effect size of Cohen criteria (G Power version 3.1.9.2, Heinrich-Heine-Universität Düsseldorf), taking alpha= 0.05 and 80% and based on the previous study [28], a minimum sample size of 30 (10/pH group) was calculated. The tested teeth (n = 33) were collected from pediatric dental clinics at Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. The teeth were carefully chosen to meet the inclusion criteria, i.e., primary posterior teeth with no carious or superficial enamel caries, free from cracks or developmental defects, and not restored previously. The selected teeth were sectioned from the cemento-enamel junction using an ortho-stripping disk (DynaFlex Corp., Chennai, India), and the sectioned coronal parts of teeth were stored in normal saline.

Specimen Preparation

Fluoride-free pumice and low-speed handpiece (KaVo Dental, Biberach, Germany) were used to clean all surfaces of the teeth included in this study. An occlusal cavity (4 mm ×4 mm height and width) was prepared in enamel and dentin part in each tooth; then the crown was cut at CEJ level and cutting the crown to 3 slides horizontally using a standard # 330 diamond bur (Brasseler USA, Savannah, GA, USA) with high-speed handpiece (KaVo Dental, Biberach, Germany) (Figure 1). Then 38% SDF - Advantage Arrest Silver Diamine Fluoride 38% (Elevate Oral Care LLC, West Palm Beach, FL USA) was applied on the prepared wall according to the manufacturer's instructions. Each sectioned specimen was immersed in an airtight polyethylene bottle containing 2 mL of respective pH media.

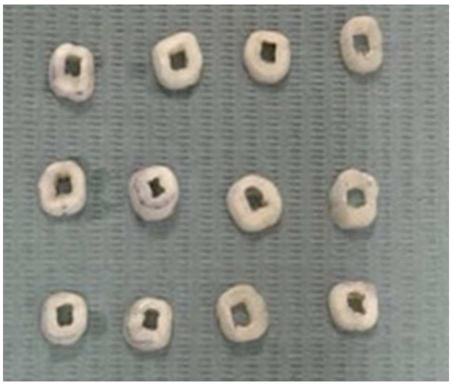

Figure 1. Specimen preparation.

Preparation of Different pH Media

In this study, one liter of alkaline, neutral, and acidic media was prepared. The alkaline pH media was prepared by mixing deionized water (Hach Lange GmbH, Düsseldorf, Germany) with 1.5 mM calcium chloride ($CaCl_2$) (PanReac AppliCham, ITW Reagents, Darmstadt, Germany), 0.9 mM sodium dihydrogen phosphate(NaH_2PO_4) (Sigma Aldrich Chemie GmbH, Steinheim, Germany), and 150 mM potassium chloride (KCl) (Techno Pharmchem, New Delhi, India), Tris buffer 20 Mm (Sigma-Aldrich Inc., St. Louis, USA), and 0.02% sodium nitrate (NaN_3) (Research-Lab Fine Chem Industries, Mumbai, India) were added, and pH was adjusted to 8-9 by the addition of dilute hydrochloric acid (HCl) (Honeywell Specialty Chemicals Seelze GmbH, Seelze, Germany).

The neutral pH media was prepared like the alkaline pH media, and the pH was adjusted to 6-7 by adding diluted HCl. The acidic pH media was prepared by mixing 2.0 mM $CaCl_2$, 2.0 mM NaH_2PO_4, 75 mM acetate buffer ($NaCH_3COO$) (Techno Pharmchem, New Delhi, India), and 0.02% NaN_3.Drops of diluted HCl and diluted sodium hydroxide (NaOH) (Emsure; Merck KGaA, Darmstadt, Germany) were added to adjust pH to 4-5 [29].

The pH of the solutions was monitored using a pH test strip, immersing it directly into the solution and keeping it in contact with the solution for 5 seconds. The strip was visually compared to the chart provided with the stripes. The samples in each pH were incubated at 37°C in each media for days 1, 2, 7, 15, and 30. At each time interval, pH media was collected for the fluoride release analysis and replaced with fresh pH media in each tube (Figure 2).

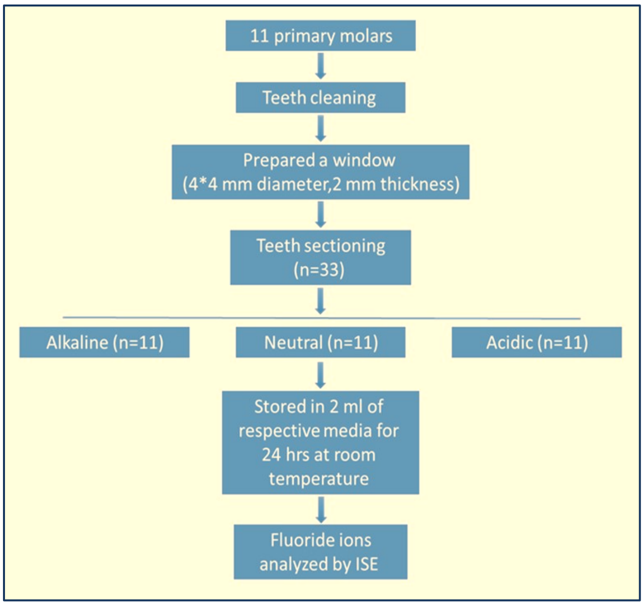

Figure 2. Flowchart illustrating the study methodology.

Fluoride Release Analysis

The fluoride ion selective electrode (ISE) (Orion Dual Star pH; Thermo Fisher Scientific Inc., Waltham, MA, USA) was connected to an ISE meter to measure fluoride ions at days 1, 2, 7, 15, and 30 under normal atmospheric conditions. The fluoride ISE was calibrated using three standard fluoride solutions (Hach Lange GmbH, Düsseldorf, Germany) at 0.1, 1.0, 10.0, and 100.0 mgL-1 concentration. After each specific interval, the specimens were removed from stored pH media and stirred continuously with 1mL of deionized water mixed with an equal amount of total ionic strength adjustment buffer (TISAB III) (Thermo Fisher Scientific Inc., Waltham, MA, USA). The buffer solution was used for washing the discs, which were later dried with absorbent paper and then re-incubated in a new 2 mL of fresh pH media. A magnetic stirrer was placed in a plastic vial with the previous respective media. The dipped electrode into the solution recorded the fluoride concentration in parts per million (ppm).

Statistical Analysis

Data analyses were performed using SPSS-20.0 (IBM Corp., Chicago, IL, USA). Numerical data based on measurements of fluoride release under the exposure to the three-pH media were presented as means ± standard deviations (SD). These numeric variables were explored for the test of normality by using the Kolmogorov-Smirnov test, which revealed a non-Gaussian (non-normal) distribution. A non-parametric Kruskal Wallis test was performed to compare mean fluoride release among three media at various time intervals. A post-hoc Mann-Whitney U test was used to compare mean fluoride release between each of the two media types. A non-parametric Wilcoxon sign rank test was used to compare mean fluoride release at various time intervals within each media. P-value ≤0.05 was considered a statistically significant difference of means.

Results

The mean fluoride release under the exposure of an acidic medium showed a significantly persistent decrease in fluoride release from day 1 to day 30 (p=0.001) compared to neutral and alkaline media. However, a non-significant difference was observed in mean fluoride release between neutral *vs.* alkaline media at all time intervals (p>0.05).

The mean fluoride release within each media on days 1, 2, 7, 15, and 30 was significant in neutral and acidic media (p<0.001). In contrast, a non-significant difference in mean fluoride release on days 2 and 7 was observed in alkaline media (p=0.875). However, significant decreases were seen on days 15 and 30 (p<0.001) using the Wilcoxon sign rank test, as presented in Table 1 and Figure 3.

Table1. Fluoride release (ppm) at various time slots under the exposure to the three different media.

Fluoride Release	Media						p-value
	Neutral		Alkaline		Acidic		
	Mean	SD	Mean	SD	Mean	SD	
Day-1	23.17	6.780	18.92	4.188	8.83	2.758	<0.001
Day-2	12.82	2.601	9.50	2.324	4.89	1.615	<0.001
Day-7	10.18	4.265	9.66	3.568	1.50	0.722	<0.001
Day-15	5.64	3.116	6.34	3.131	0.60	0.157	<0.001
Day-30	3.32	1.839	4.18	2.472	0.47	0.162	<0.001

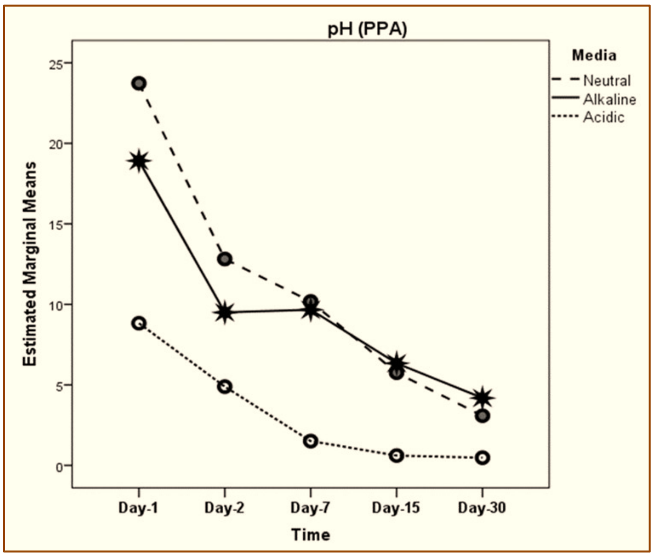

Figure 3. Comparison of mean fluoride release (ppm) at various time slots under different pH media.

Using the Kruskal-Wallis test, fluoride release was significantly different at various time durations among the three media types (p=0.001). There was a significant difference in mean fluoride release between acidic *vs.* neutral media and acidic *vs.* alkaline media (p=0.001), with non-significant differences between neutral *vs.* alkaline media at all time intervals (p>0.05) (Tables 2 and 3 and Figures 3 and 4). In addition, a significantly persistent lower mean fluoride release under exposure of all three media from day 1 to 30, except alkaline media non-significant from day 2 to 7 (p=0.875). Therefore, the results of our experiment showcase SDF's significant in-vitro efficacy in its potential preventive characteristics against various bacteria, especially in acidic media.

Table 2. Comparison of cumulative fluoride release (ppm) at various time slots under the exposure to the three-pH media.

Fluoride Release	Neutral	Alkaline	Acidic
Day-1	23.17	18.92	8.83
Day-2	35.99	28.42	13.72
Day-7	46.17	38.08	15.22
Day-15	51.81	44.42	15.83
Day-30	55.13	48.61	16.31

Table 3. Difference in Fluoride release within the three media during multiple time intervals.

Fluoride Release	Media (I)	Media (J)	Mean Difference (I-J)	p-value
Day-1	Neutral	Alkaline	4.250	0.103
	Neutral	Acidic	14.339*	0.000*
	Alkaline	Acidic	10.089*	0.000*
Day-2	Neutral	Alkaline	3.318*	0.003*
	Neutral	Acidic	7.927*	0.000*
	Alkaline	Acidic	4.609*	0.000
Day-7	Neutral	Alkaline	0.5152	0.924
	Neutral	Acidic	8.6727*	0.000*
	Alkaline	Acidic	8.1576*	0.000*
Day-15	Neutral	Alkaline	-0.7000	0.787
	Neutral	Acidic	5.0326*	0.000*
	Alkaline	Acidic	5.7326*	0.000*
Day-30	Neutral	Alkaline	-0.8583	0.484
	Neutral	Acidic	2.8477*	0.002*
	Alkaline	Acidic	3.7061*	0.000*

*Statistically significant.

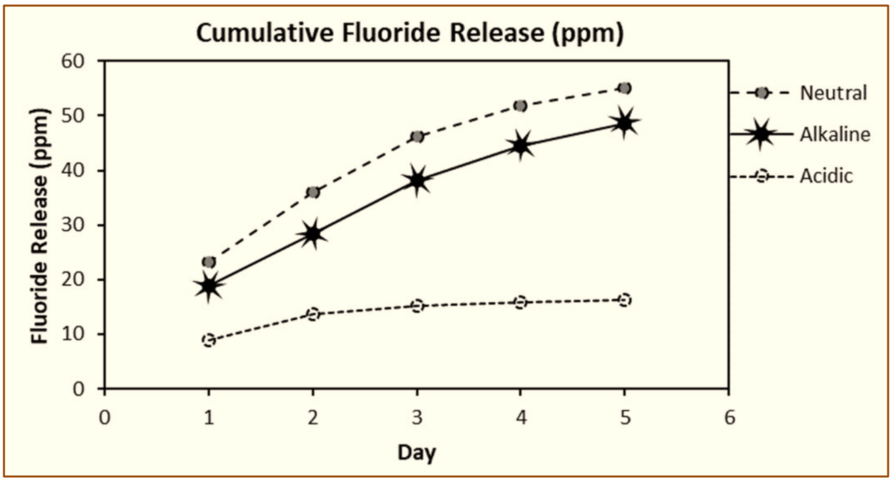

Figure 4. Comparison of cumulative fluoride release (ppm) at various time slots under different pH media.

Discussion

SDF has been used for many years to prevent caries and arrest material in primary and permanent dentition [30,31]. In this in vitro study, the effect of different pH media on the concentration of fluoride release from SDF has been investigated. The maximum amount of fluoride release was observed in an alkaline medium on day 30. Thus, this result is comparable with the null hypothesis.

The formation of a high plaque index and a reduction in the pH or acidogenic ability causes decay. The influence of pH in demineralization indicates that decreasing the pH may favor the cariogenic process. Reversion of this tooth demineralization can be induced when the pH of the oral cavity is found to be at 5.5 [32]. SDF is known to prevent and control dental caries effectively and is used in different concentrations in children with high caries risk. The topical application of SDF is a cost-effective, user-friendly, and non-invasive approach in caries management [33]. Fluoride is an essential component of SDF to facilitate remineralization and hardening of the tooth structure. It is reported that the SDF is associated with a definite increase in fluoride levels [34].

The present study evaluated the effect of different pH media on the concentration of F release of 38% silver diamine fluoride for one month. Fluoride release was the highest on day one and started to decrease gradually, with this reduction being the least in acidic media. These data indicate that the product delivers the advertised concentration of fluoride when first opened; however, the concentrations decrease after opening [29]. Previous research has shown that SDF application results in the formation of CaF_2, resulting in the formation of fluorapatite crystals, which are more resistant to acidic dissolution [35].

The highest fluoride concentration of SDF in all storage media occurred 24 hours post-application and is known as the "burst effect". These findings are similar to various restorative materials such as glass ionomer cement, resin-modified glass ionomer cement, compomer, and composite resin, where the "burst effect" occurred 24 hours after post storing in different pH media [29,36,37]. In this study, the amount of fluoride released was influenced by the storage medium. The mean fluoride release value showed a significant difference between acidic and alkaline media at all intervals. In acidic media, a minimum fluoride release value was observed, whereas maximum fluoride release was observed in alkaline media. The possible reasons for the minimum fluoride release value in the acidic media could be the partial demineralization of the crystals that might attract and absorb fluoride to the crystal surface and the crystal behaving like fluorapatite. Meanwhile, in alkaline, the crystals are not affected, and there is no need for fluoride absorption; therefore, the fluoride content is high in the alkaline media [38,39]. Also taken into consideration, the reduction of fluoride release occurred in acidic media as a result of using competitor anions in preparing acidic media like chloride, nitrate, sulfate, and phosphate. In addition, phosphate and sulfate have larger ionic radii and higher surface charger densities than fluoride, resulting in a slight decrease in fluoride release (40). In contrast, previous investigations of fluoride release from glass-ionomer cement and resin-based composites showed more fluoride release with acidic media [29,36].

It is reported that annual application of either SDF solution or high fluoride-releasing glass ionomer can arrest active dentine caries with a rate of 79% and 82%, respectively. Increasing the frequency of application every six months can increase the caries arrest rate of SDF applications by 91% [41]. In the present study, the mean fluoride release at days 1, 2, 7, 15, and 30 was significant in neutral ($p<0.001$). This result is comparable to a study conducted to analyze the amount of fluoride released from SDF in artificial saliva at a pH of 7 on days 1, 7, and 14 [42].

A significantly persistent decrease in mean fluoride release was observed under exposure to all three media from day 1 to 30, except alkaline media, which was non-significant from day 2 to 7 ($p=0.875$). In contrast, a study reported that the percentage of fluoride ions released following the application of SDF within 60 days was increased [43]. However, the results of this study contradict previous studies, which found that ammonia ions bond with silver ions, forming a stable complex ion called the silver diamine ion $[Ag(NH3)2]^+$ [44].

Based on previous studies, fluoride in low concentration (up to 1 ppm) in a solution significantly protects against demineralization of teeth structure [45,46]. The same was obtained in this study, as SDF in all tested media during the 30 days of trial effectively reduced the dissolution of tooth structure.

One of the limitations of this study is that it was conducted under in vitro conditions, which cannot replicate the oral cavity's complexity regarding temperature and other factors. However, it is felt that the result from the present investigation will enable pediatric dentists and other dental professionals to know and critically analyze the materials based on their fluoride-releasing properties.

Conclusion

The fluoride release concentration of 38% SDF is under the influence of pH storage media. All the specimens released fluoride ions during the entire experimental period. Abrupt fluoride release was found in all groups; however, a continuous release pattern was observed in all media. Overall, the neutral group showed the highest fluoride release compared to others. The release concentration from all groups is sufficient to restrict the caries.

Authors' Contributions

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NA	https://orcid.org/0009-0009-3144-9787	Conceptualization, Methodology, Investigation and Writing - Original Draft.
MAG	https://orcid.org/0000-0001-5590-526X	Data Curation and Writing - Review and Editing.
MSI	https://orcid.org/0000-0001-8461-3354	Writing - Review and Editing.
ASK	https://orcid.org/0000-0002-2165-800X	Writing - Review and Editing and Supervision
BOG	https://orcid.org/0000-0001-7593-1887	Conceptualization, Formal Analysis, Writing - Review and Editing and Supervision

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

Financial Support

None.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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