Fluoride and silver concentrations of silver diammine fluoride solutions for dental use

Abstracts

Background: *Silver diamine fluoride (SDF) in high fluoride concentration at 38% has been shown to be effective in arresting caries in children. The stability of the high concentration of fluoride in commercial silver fluoride preparations is an important safety issue for use in paediatric dentistry.*

Aim: *To determine the short-term stability of free fluoride ion concentrations and acidity (pH values) of three commercially available SDF solutions over time.*

Design: *Three SDF products for caries control were studied: Cariestop-12%, Cariestop-30% and Saforide-38%. Their expected fluoride ion concentrations were 14,200 ppm, 35,400 ppm and 44,800 ppm, respectively. The free fluoride ion concentrations were determined with an ion-selective electrode. The acidity was determined with a pH electrode. The measurements were performed when open and at 7 and 28 days of storage at 4°C. Five bottles of each product from the same lot number were measured.*

Results: *The mean free fluoride ion concentrations of the freshly opened bottles were 12,525±450 ppm, 13,200±2,060 ppm and 55,800±2,536 ppm, respectively. The mean pH values were 9.4±0.1, 10.4±0.1 and 10.2±0.2, respectively. Statistical testing (ANOVA) found no significant change (p>0.05) in the free fluoride ion concentrations or the acidity was detected after 7 or 28 days.*

Conclusion: *All of the SDF solutions tested were alkaline. The free fluoride ion concentrations of Cariestop-30% and Saforide-38% were considerably different. The fluoride ion concentrations and acidity of all three products demonstrated a short-term stability over 28 days.*

Introduction

Silver diamine fluoride (SDF) is a cost-effective agent used for caries control, and appears to conform to the World Health Organization's Millennium Goals and the US Institute of Medicine's criteria for 21st-century medical care. Clinical trials have shown that SDF prevents and arrests coronal caries in primary teeth in preschool children[1] and in permanent teeth in older children.[2] SDF has also been shown to be effective in the prevention of root caries in the elderly.[3] Various regimens, including one-off, semi-annual and annual applications, are prescribed.[1, 2, 4] Clinical trials reported in the literature in English shown that the most commonly used agents contain a high concentration (38%) of SDF.[1, 2] A 38%-SDF product has been accepted as a therapeutic agent by the Central Pharmaceutical Council of the Ministry of Health and Welfare of Japan for dental treatment for half a century.[5]

Using products with a lower concentration of SDF has been suggested, but the results of clinical trials are inconsistent.[6, 7] Yee et al. [7] reported that a one-off SDF application was effective in arresting caries if the concentration was 38%, but not if it was 12%. Thirty-percent SDF is also available on the market. Clinical studies reported no significant side-effects of its use on children.[2, 6, 7] Transient mild gingival and mucosal irritation may occur.[2] SDF displays anti-microbial activity against cariogenic mono-species biofilm of *Streprococcus mutans* or *Actinomyces naeslundii* formed on dentine surfaces.[8]

In addition to the antimicrobial and caries arresting effects of SDF, the stability of the reagent is also crucial in arresting the progress of caries. However, no studies have examined the stability of SDF at different concentrations. SDF is often used in very high concentration in caries management to arrest dental decay. It is important not to over-zealously applied SDF in the caries lesion in particular on young children. Despite the amount used would be very small, a laboratory study by Gotjamanos and Orton raised the concern of dental fluorosis when it is used in young children.[9] Gotjamanos and Orton found that commercial preparations of 40% neutral silver fluoride had a significantly higher than expected concentration of fluoride[9], but there are no reports of the free silver and fluoride ion concentrations of commercially available SDF solutions. This study thus aimed to investigate the exact concentrations and stability of different concentrations of commercially available SDF products, which are not the same product studied by Gotjamanos and

Orton.[9] The objectives of the study were to measure the free fluoride and silver ion concentrations (mg/L) of three commercially available SDF solutions, to measure the acidity (pH value) of the three solutions and to determine the short-term stability of the free fluoride ion and free silver ion concentrations and the acidity of the three products.

Materials and methods

Three common commercially available SDF products for caries control were studied: Cariestop-12%, Cariestop-30% and Saforide-38% (Table 1). Their SDF concentrations were expected to be 12%, 30% and 38%, respectively. Their lot numbers were 7505 94/11, 7506 651/10 and 909 PA; and expiration date were Feb 2013, Oct 2012 and Sep 2012, respectively. Five bottles of each SDF product with the same lot number were purchased for assessment in July 2011. The solutions were diluted with deionised water (dilution factor 1:100) before undergoing fluoride and silver testing. The free fluoride and silver ion concentrations were measured immediately (Day 0), at 7 days (Day 7) and at 28 days (Day 28) after the bottles were opened. After opening, the SDF products were stored at 4^{o}C in a freezer.

Determination of the Free Fluoride Ion Concentration

Free fluoride ion concentration was determined by fluoride ion-specific electrode direct read method.[10] Ten millilitres of pH-adjusted (pH 6.0) total ionic strength adjustment buffer was added to 10 ml of the diluted SDF solutions to increase their ionic strength. The ion-selective electrode (PF-1, Shanghai Precision and Scientific Instrument Co Ltd, Shanghai, China) was tested for accuracy using standardised 100, 200, 400 and 600 μg/mL fluoride solutions. The electric potential of each solution was measured using the electrode on a potentiometer (model PHS-3C, Shanghai Precision and Scientific Instrument Co Ltd, Shanghai, China). A standard curve was produced by plotting a line graph of fluoride concentration (X-axis) against electric potential (Y-axis). From the measured electric potential of the test sample, the fluoride concentration was determined using the standard curve. Two measurements were performed on the sample from each bottle, and the average value was recorded as the reading from that bottle. A mean value was calculated from the five readings of the five bottles studied.

Determination of the Free Silver Ion Concentration

The silver ion concentration was measured using a CCD simultaneous inductively coupled optical emission spectrometer (Varian 720-ES, Varian Inc., Santa Clara, USA). [11] The inductively coupled optical emission spectrometer 720 operated at 1 kW (RF power) with a plasma flow rate of 15L/min. Five millilitres of the diluted test solution was converted to a fine spray by a nebuliser and mixed with argon in a spray chamber. The sample was added to the plasma and instantly excited by the high temperature, which allowed the silver content of the solution to be measured with an emission line of 328 nm. Two measurements were performed on each sample, and the average value was recorded for analysis.

Measurement of acidity (pH)

The pH value of the test solutions was determined using a PHM 240 pH electrode (Radiometer Analytical, Lyon, France) at room temperature. Before the measurement, the electrode was thoroughly rinsed with potassium chloride. An electrical potential difference was produced when the electrode was placed into the test solution. This was then compared with the potential difference of the reference electrode with a pH meter.

Statistical analyses

Data analyses were conducted using the SPSS software version 19.0 (IBM Corporation, Armonk, USA). The data were tested for normality using the Shapiro-Wilk test. A one-way ANOVA was used to compare the concentrations of silver and fluoride ions and the pH values measured when the bottles were open and at day 7 and 28. The cut-off level for significance for all of the analyses was 5%.

Results

The results for the free fluoride and silver ion concentrations and pH values of the three commercial products are given in Table 2. The mean free fluoride concentration of the freshly opened 12% SDF was 12,525ppm, which was 12% lower than the expected 14,500 ppm. The mean free fluoride concentration of the freshly opened 30% SDF was 13,200 ppm, which was only 37% of the expected 35,400 ppm. The mean free fluoride concentration of the freshly opened 38% SDF was 55,800 ppm, which was 25% higher than the expected 44,800 ppm. The variations in the measured and expected free silver ion concentrations were smaller than those of the fluoride ion concentrations. The free silver ion concentrations of the freshly opened bottles of 12%, 30% and 38% SDF (Cariestop-12%, Cariestop-30% and Saforide-38% were 79,000 ppm, 213,000 ppm and 249,000 ppm respectively. These values were 9%, 106% and 98% of the expected silver ion values of 80,000 ppm, 200,000 ppm and 255,000 ppm. The pH values of all three products were alkaline and had a pH of around 9 to 10. The pH values of the Cariestop-12% and Cariestop-30% were 9.4 and 10.4, respectively, which were higher than the value of 8.5 claimed by the manufacturer. The pH of Saforide-38% was not stated by the manufacturer, but was 10.2 when freshly opened. The pH values of the three products were stable and showed only a slight variation of 0.2 after 7 and 28 days. The free fluoride and silver ion concentrations of the three products showed no statistical significant change after 7 or 28 days.

Discussion

Silver ions have been found up to the coronal pulp after the use of 40% silver fluoride solution for the atraumatic treatment of cavities close to the pulp.[12] The silver ion deposits remained localised at the base of the cavity, so it is conceivable that a considerable amount of concentrated fluoride may be released from the silver fluoride. Afonso and Gotjamanos[12] expressed concern that the released fluoride could pass through the dentine to the dental pulp, enter the general circulation and contribute to dental fluorosis. Their subsequent study of the fluoride content of silver fluoride using an ion-selective electrode found that a sample of 24 commercial preparations of 40% neutral silver fluoride in Australia had a significantly higher concentration of fluoride (up to 120,000 ppm) than the expected level of 60,000 ppm.[13] In another laboratory study, Gotjamanos and Orton [14] used an ion-selective electrode and ion chromatography and found mean fluoride ion concentrations that were considerably higher than the expected 60,000 ppm in 40% neutral silver fluoride solution. The additional presence of silver difluoride and hydrofluoric acid resulting from the manufacturing process was suggested as an explanation for the much higher than expected levels of fluoride ions. The authors concluded that the 40% neutral silver fluoride solution that is commercially available in Australia may be too concentrated for use in treatment, and carried a risk of toxicity leading to dental fluorosis when used on young children. However, the Dental Services of the Health Department of Western Australia, in response to the study of Gotjamanos and Orton, carried out an investigation and found no evidence to support the view that the proper use of the neutral silver fluoride solution would cause dental fluorosis. [15] The Health Department reported that they had sought opinions from various scientists, including Ole Fejerskov, all of whom discounted the possibility of dental fluorosis formation from a single minute application of fluoride to a caries lesion. In this study, the measured fluoride ion concentration of Saforide-38% of up to 60,000 ppm was much higher than expected. A single minute application at this concentration is unlikely to cause dental fluorosis, but caution should be taken to avoid frequent application or the application of a large quantity, particularly in very young children.

The measurement of fluoride concentration in aqueous solutions reported in literature is not standardised. [16] It can be performed with the ion chromatographic method or the ion-selective electrode method. Ion chromatography is commonly used to monitor water quality and to determine ionic species with respect to corrosive ions.[17] For the determination of lower levels of fluoride ions, the pre-concentration of the sample is necessary, but this has several disadvantages. [17] The ion-selective electrode method is commonly used to measure fluoride concentration, and is popular because the aqueous matrices are easy to analyse and sample preparation is simple.[18] Martinez-Mier et al.[16] proposed a gold standard for fluoride analysis and they recommended direct and micro-diffusion methods using fluoride ion-selective electrode. This multi-steps method was used to analyses fluoride content of food samples consumed in the United States by the U.S. Department of Agriculture.[19] The U.S. Department of Agriculture used a simpler fluoride ion-specific electrode direct read method for clear liquids. This direct read method was used in this study because the SDF products tested are all colourless clear liquids. Hence, an ion-selective electrode was chosen to analyse the fluoride ion levels in this study. Ion-selective electrode method has the advantages of such as short response time and non-contaminating. There are a number of limitations. The precision ion-selective electrode is not high. Interference can occur by other ions. Moreover, the electrodes can be fouled by proteins or other organic solutes, and respond to the activity of uncomplexed ion. van den Hoop et al.[20] studied the fluoride concentration of rainwater and found the concentrations measured by ion chromatography to be significantly higher than those measured by an ion-selective electrode. They suggested that the differences may be due to the presence of metal cations. It is noteworthy that fluoride binds to metal cations, which means that only a fraction of the total fluoride concentration is determined in many instances. Total ionic strength adjustment buffer was added to the samples in this study to overcome this problem. It contains a metal complexing agent that eliminates the cationic binding of fluoride ions, allowing them to be released as free ions and thereby minimising the variation in fluoride ion concentration measurements.

Inductively coupled optical emission spectrometry is a type of emission spectroscopy and analytical technique used for the detection of trace metals in aqueous solutions. It uses inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths that are characteristic of a particular element.[21] At high plasma temperatures, all compounds are completely dissociated and are in an excited state. They then emit characteristic lines in visible and ultraviolet light.[22] This study employed inductively coupled optical emission spectrometry to determine the silver ion concentration because of its high sensitivity and relative freedom from matrix effects.[23] It is noteworthy that although inductively coupled optical emission spectrometry generally exhibits good accuracy and precision, a variety of factors can affect the measurement results. Measures to avoid such influences while preparing samples for analysis include the precise dilution of the original samples, minimisation of the time taken to prepare the samples preparation and the proper storage of the solutions.

This study assessed three SDF solutions commonly used to arrest caries with concentrations of 12%, 30% and 38%. It is noteworthy that at present these products are not available in countries such as USA, Canada and UK. Saforide (38% SDF) is one of the most widely used SDF products. [1, 2, 23] The results showed all three products to have stable fluoride and silver concentrations at the three time points. Although neutral silver fluoride is much more soluble in water than other silver halides, it forms a colourless cubic crystal. SDF is a complex in which ammonia ions combine with silver ions to produce a complex ion called diamine-silver ion $[Ag(NH_3)_2]^+$. The formation of diamine-silver ions is a reversible reaction. However, the complex is very stable, with an equilibrium position that lies within the diamine silver ions.[23] Many silver ions are removed from the solution, which produces a smaller value less than the solubility product, but there is less precipitation of neutral silver fluoride. This may account for the stability of the SDF solutions over time.

Silver compounds have been used to manage dental caries for decades. Reviews found silver compounds in particular silver diamine fluoride is effective in arresting active caries.[4, 23] The mechanism of action still needs to be confirmed. However, silver ion has anti-bacterial effects, and thus some researchers suggested that it interfere bacterial cell membranes and cytoplasmic enzymes and inhibit bacterial DNA replication. Silver ions might also exert its effect directly on the caries lesion by inhibition of the demineralization process. Theoretically, the molar concentration of the silver in SDF equals that of the fluoride (formula: $Ag(NH_3)_2F$). The ratio of the mass concentration of silver to fluoride should thus equal the molecular weight ratio of silver to fluoride. The expected concentration of silver and fluoride for each SDF solution was calculated from the concentrations provided by the manufacturer. It is noteworthy that the SDF products tested in this study were belonged to the same lot. It is uncertain if this variation of fluoride concentration is an incidental finding. The results were sent to manufacturers by e mail. Interestingly, the fluoride concentration of Saforide-38% was 25% higher than the expected fluoride level (55,000 ppm vs. 44,800 ppm). One explanation for this result is that both silver fluoride and silver difluoride are formed from SDF solutions through the dissolution of silver oxide or silver carbonate in hydrofluoric acid.[9] The free fluoride ion concentration of Cariestop-30% was only 37% of the expected concentration (13,200 ppm vs. 35,400 ppm). According to information sheet provided by the manufacturer, Cariestop-30% contains fluoridic acid, silver nitrate and ammonia hydroxide. Fluoridic acid is a double fluoride, consisting essentially of a solution of boron fluoride in hydrofluoric acid. Fluoridic acid has one free fluoride ion. The remaining three fluoride moieties bind with boron covalently, and may not be free to be detected by an ion-selective electrode. If the fluoride moieties cannot form an SDF complex through chemical reaction, they will most probably remain bound to the boron covalently and will not be detectable. This may account for the lower than expected fluoride concentration value of Careistop-30. Cariestop-12% has the same composition as Cariestop-30%, but a lower concentration of SDF. This study found the concentration of Cariestop-12% to be similar to the expected concentration. This may be because fluoridic acid is more readily formed in solutions with a higher concentration of SDF. The lower fluoride concentration of 12% may affect the effectiveness of caries arrest, but no clinical studies have compared the difference in effectiveness of 38% SDF and 30% SDF.

Conclusion

The concentration of free fluoride ions in the freshly opened Cariestop-30% was only 37% of the expected value, whereas that of Saforide-38% was 25% higher than expected. In this study, the opened SDF bottles were stored at 4^{o}C. The free silver and fluoride ion concentrations of all three products displayed no statistically significant change after 7 or 28 days. All of the SDF solutions tested were stable and alkaline, with a pH of around 9 to 10 after 28 days.

Why this paper is important for paediatric dentists

- Silver diamine fluoride (SDF) is an effective fluoride agent that is used in high concentration to arrest caries in children.
- There is concern that commercial products containing SDF may have an abnormally high concentration of fluoride, but no study has been performed to assess the amount of free silver and fluoride ions in commonly available commercial SDF products.
- This study provides useful information on the short-term stability of free fluoride and silver ion concentrations and acidity of SDF.

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Table 1 SDF commercial products used in this study

Product	Manufacturer	Concentration	Ingredients
Cariestop-12%	Biodinamica, Brazil	12%	Fluoridic acid, sliver nitrate, ammonia hydroxide, deionised water
Cariestop-30%	Biodinamica, Brazil	30%	Fluoridic acid, sliver nitrate, ammonia hydroxide, deionised water
Saforide-38%	Toyo seiyaku kasei, Japan	38%	Silver diamine fluoride

Table 2 Fluoride and silver ion concentrations and acidity of the silver diamine fluoride solutions over time

	Cariestop-12% (N=5)			Cariestop-30% (N=5)			Saforide-38% (N=5)		
	F (ppm)	Ag(ppm)	pH	F (ppm)	Ag(ppm)	pH	F (ppm)	Ag(ppm)	pH
Expected value	14150	80170	8.5	35400	200400	8.5	44800	253870	Not mentioned
Day 0	12525±450	79112±5559	9.4±0.1	13200±2060	213000±8124	10.4±0.1	55800±2536	248950±6345	10.2±0.1
Day 7	11206±1410	79012±5467	9.3±0.0	11920±597	221400±8111	10.5±0.1	55900±1039	240600±8561	10.2±0.1
Day 28	10985±880	78740±3842	9.3±0.1	10760±1594	215200±8871	10.4±0.1	53760±676	241600±7402	10.0±0.1
p value	0.09	0.99	0.34	0.08	0.30	0.97	0.11	0.20	0.15