

Analysis of Commercial Fluoride-containing Mouthwashes for Children Using Nuclear Magnetic Resonance Spectroscopy

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Most fluoride-containing mouthwashes for children have flavors and fragrances added to them, so you need to be careful about swallowing accidents. It is also necessary to check the amount of fluoride remaining in the oral cavity after using them. Therefore, this study compared whether the total fluoride (TF) of three types of children's mouthwash commercially available in Korea is compatible with the standards indicated by manufacturers. In addition, the amount of fluoride remaining in the saliva of the oral cavity after using mouthwash was confirmed through Fluorine (¹⁹F) Nuclear magnetic resonance (NMR) spectroscopy. As a result, even fluoride in Garglin Kids Care was marked as 226.1 ppm but detected TF was at an average of 455.818, which was about 102 % more. The fluoride remaining in saliva after 1 minute of using mouthwash was 0.0144 % for 2080 Kids, 15.4477 % for Chikachika, and 0.0015 % for Garglin Kids Care. After 2 minutes of using mouthwash, very little fluoride was present in the oral saliva in all three types of mouthwashes. These results confirmed that mouthwash containing fluoride for children does not affect the human body, such as toxicity. Therefore, the safe use of mouthwashes can lead to the improvement of oral health in children.

Keywords : Nuclear magnetic resonance (NMR) spectroscopy, mouthwash, fluoride, safety

1. Introduction

The recent COVID-19 outbreak has caused a worldwide pandemic, and the SARS-CoV-2 virus has been found in 91.7 % of saliva samples from patients at an early stage of infection and is considered a potential route of COVID-19 transmission [1, 2]. Saliva containing the SARS-CoV-2 virus is produced through coughing, sneezing, breathing, and conversation, making about 40,000 saliva droplets in a single cough, and can travel several meters in the air, promoting infectious diseases [3]. In general, splashes of saliva or water droplets can contaminate an area 3 feet in diameter, whereas the aerosol generated from there can lead to sustainable contamination over large areas [4]. To reduce the risk of SARS-CoV-2 transmission, mouthwash is recommended as an effective measure [5].

Mouthwash not only reduces bacteria in the mouth but

also prevents periodontal disease, prevents dental caries, remineralizes early caries, reduces bad breath, and alleviates dentin hypersensitivity. Because the active ingredient of mouthwash directly reduces the number of bacteria, it is helpful for oral health management of children with disabilities and children who have difficulty in brushing [6]. Children under the age of 6 prefer sweets or drinks containing a large amount of sugar compared to other age groups, and the incidence of dental caries is high due to the high frequency of snack intake [7]. Therefore, proper use of fluoride-containing mouthwash is crucial to prevent dental caries in children. Fluoride compounds in oral gargles have been used in oral gargles since the 1940s because they have the effect of remineralizing teeth to prevent dental caries and inhibit the activity of bacteria that cause periodontal disease. Sodium fluoride and tin fluoride are used as fluoride compounds. Even tin fluoride has the side effect of forming discoloration on teeth but is stable when used temporarily [8]. The United Kingdom restricts fluoride-containing mouthwash at less than 600 ppm for children under six years of age, while Greece and the Netherlands recommend less than 500 ppm [9]. As

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mentioned before, daily use of mouthwash containing fluoride has a preventive effect on dental caries [10]. However, if used without considering age, it can lead to irreversible results such as enamel fluorosis of the anterior region, so excessive fluoride consumption should be avoided [11]. In particular, it is necessary to check the amount of fluoride remaining in the mouth after using the fluoride gargle because mouthwash for children has added flavors and fragrances, and children often swallow the mouthwash intentionally.

Methods for measuring the amount of fluoride in the oral cavity include isotachopheresis [12], ion chromatography [13], gas chromatography [14], and a measurement method using a fluoride ion electrode [15]. The method using the fluorine ion electrode has been widely used due to its excellent simplicity and accuracy. But there is a difficulty in adjusting the overall ionic strength and buffering because it responds not to the concentration itself but the activity [16]. Accordingly, Mohammed *et al.* [17] used Nuclear Magnetic Resonance Spectroscopy (NMR) to analyze the fluorapatite crystal structure of enamel. Nam and Han [18] studied the quantitative evaluation of fluoride by applying Fluorine (^{19}F) NMR taking advantage of observing fluorine nucleus resonance without background problems. This nuclear magnetic resonance method is mainly applied to structural analysis in the engineering field [19]. It is a very effective method because the fluoride atom has a small radius, strong electronegativity, and high binding energy compared to other atoms [17].

Therefore, this study measured the total fluoride (TF) of mouthwash for children containing fluoride sold in Korea using ^{19}F NMR and confirmed the standards indicated by manufacturers to ensure that the amount of residual fluoride in the mouth is safe for children when using fluoride-containing mouthwash.

2. Materials and Methods

2.1. Sample preparation

In this experiment, we purchased three products common commercially available products classified as mouthwashes for children containing fluoride that are readily available

in Korea. Table 1 provides information on commercially available mouthwashes for children used in this study. All products had more than six months of expiration date left. For the blind test, we coded the mouthwashes alphabetically and measured the fluoride content of each mouthwash. To measure the residual fluoride, children under six years of age used 2080 Kids and Chikachika for 30 seconds and Garglin Kids Care for 1 minute, according to the manufacturer's instructions. This study was conducted on 21 children in Gangwon-do, under the age of 5 or 6, who explained the purpose and method of this study and agreed to participate. The 400 μl of the collected saliva samples 1 minute and 2 minutes after mouthwash were dissolved in deuterium oxide (D_2O) and analyzed.



Fig. 1. (Color online) FT-NMR 400 MHz spectrometer used for measuring the total fluorine content.

Table 1. Fluoride-containing mouthwashes for children used in this study.

Products	Total fluoride (ppm)	Active ingredients	Manufacturers	Codes
2080 Kids	90	Cetylpyridinium chloride Sodium fluoride	Aekyung	A
Chikachika	226.1	Sodium fluoride	Samil pharm.	B
Garglin Kids Care	226	Sodium fluoride	Dong-A pharm.	C

2.2. Fluoride analysis

An ECZR NMR spectrometer (FT-NMR 400 MHz Spectrometer, JNM-ECZ400S/L1, JEOL Ltd, Tokyo, Japan) performed the ¹⁹F NMR measurements. The spectrometer operated at 376.17 MHz, and a dedicated 5mm spinning probe was equipped with 23 °C probe temperature (Fig. 1). Typical spectral parameters for this study were 90° pulse width, 6.74 μs of relaxation delay, and 5 s of acquisition time 83.88 s. As an internal field frequency lock, a known amount of D₂O (700 μl) was added.

Using the following formula, Spectral resonance frequency (V_0), the NMR spectrometer calculated.

$$V_0 = \frac{\gamma}{2\pi} B_0$$

r = gyromagnetic ratio

B_0 = Magnetic Field strength

And Chemical shift (ppm) were used the following formula.

$$\text{Chemical shift (ppm)} = \frac{V_i - V^{ref}}{V_0} \times 1,000,000$$

V_0 = The resonance frequency of the chemical bonds that are not nuclear

V_i = The resonance frequency of each element in the

molecule

V^{ref} = The reference frequency

3. Results

Figure 2 is the analysis of the ¹⁹F NMR spectrum within the range from -122.44 ppm to -120.41 ppm. The peak of the graph confirmed the detected amount of fluoride. The fluoride content of 2080 Kids was 90 ppm, a low concentration, but the detected TF was an average of 25.956, about 29 % less. The fluoride content indicated in Chikachika was 226 ppm, but the average TF was

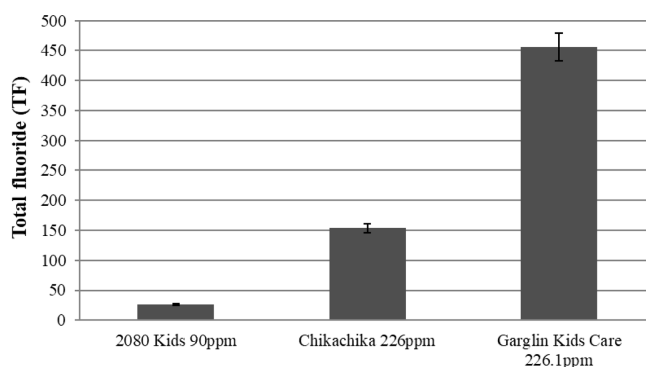


Fig. 3. Quantified TF levels of fluoride-containing mouthwashes for children by ¹⁹F NMR analysis.

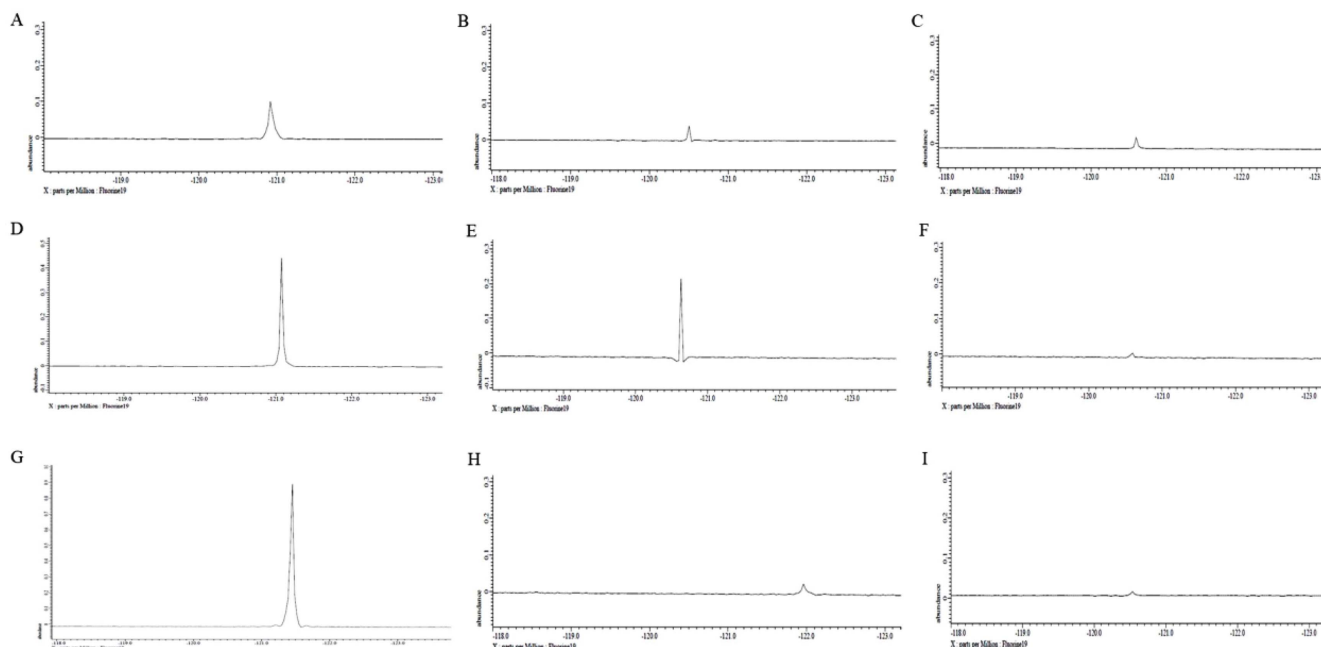


Fig. 2. Fluoride peak areas detected through NMR analysis. A; 2080 Kids 90 ppm, B; The residual amount of fluoride after 1 min of using 2080 Kids, C; The residual amount of fluoride after 2 min of using 2080 Kids, D; Chikachika 226 ppm, E; The residual amount of fluoride after 1 min of using Chikachika, F; The residual amount of fluoride after 2 min of using Chikachika, G; Garglin Kids Care 226.1 ppm, H; The residual amount of fluoride after 1 min of using Garglin Kids Care, I; The residual amount of fluoride after 2 min of using Garglin Kids Care.

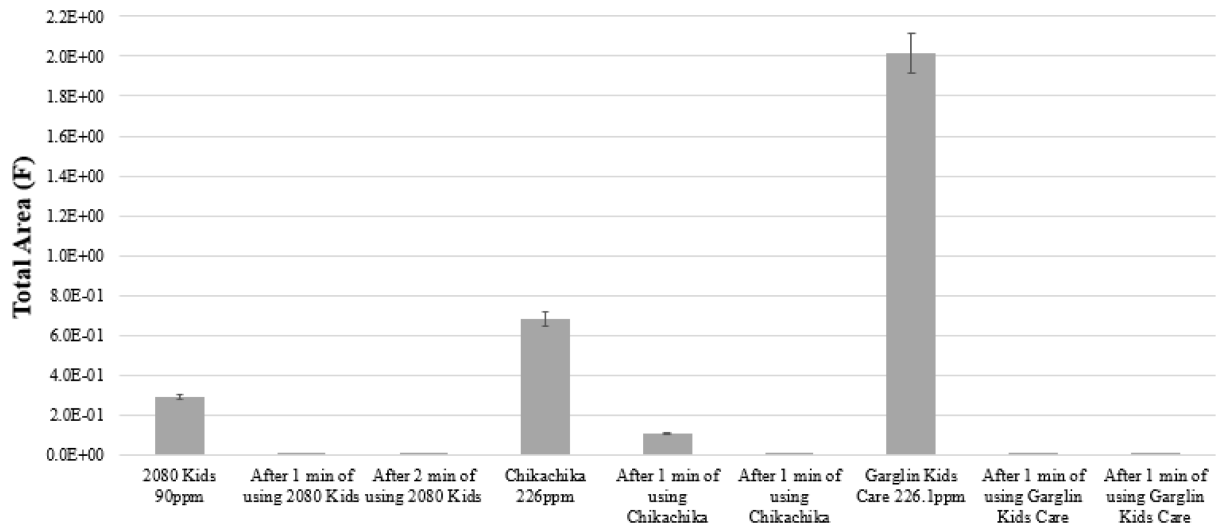


Fig. 4. Residual fluoride concentration levels after using fluoride-containing mouthwashes for children.

153.879, which was detected about 32 % less. On the other hand, in the case of Garglin Kids Care marked as 226.1 ppm, TF was an average of 455.818, which was 102 % more (Fig. 3). After 1 minute of using the mouthwashes, the result of measuring the amounts of fluoride that remained in saliva was 0.0144 % for 2080 Kids, 15.4477 % for Chikachika, and 0.0015 % for Garglin Kids Care. After 2 minutes of using each mouthwash, the residual amount was 0.0068 % for 2080 Kids, 0.0013 % for Chikachika, and 0.0004 % for Garglin Kids Care, confirming very little amount is present in the oral saliva (Fig. 4).

4. Discussion

Fluoride is a representative substance that suppresses enamel demineralization and remineralizes the demineralized tooth surface to prevent dental caries [20]. As a method of applying fluoride in the oral cavity, there are fluoride application and fluoride ionization legislation performed by experts in dentistry, but there is the inconvenience of having to visit the dentist regularly every six months. Therefore, fluoride-containing toothpaste and mouthwash easily used at home are preferred. Children under the age of 6 have difficulty brushing their teeth, so when using fluoride-containing toothpaste, fluoride may remain in the oral cavity and may even be swallowed.

In a study on the brushing habits of 500 children aged three years, Palmer and Prothero [21] reported that when brushing teeth, 23 % of children swallowed most of the toothpaste used, 29 % swallowed half, and 46 % swallowed some amount of toothpaste. Ericsson and Forsman [22] reported that 24.5 %-33.2 % of fluoride in toothpaste

remained in the oral cavity after brushing in children aged 4 to 7 years. They also reported that children under two years of age swallowed more toothpaste. Based on the 1984 standards of the American Academy of Pediatric Dentistry (AAPD) [23], children aged 3-4 years intake 0.29 mgF of fluoride in toothpaste when they use 1000 ppm of fluoride-containing toothpaste. When a child lives in a fluoride area of 0.8 mgF, the maximum fluoride limit of 0.95 mgF exceeds, which can cause chronic fluoride poisoning. Therefore, if a child uses the same 1000 ppm fluoride-containing toothpaste as adult toothpaste may cause the following problems: mottled teeth, direct ingestion of fluoride due to carelessness during brushing, and swallowing remained fluoride after rinsing. Hence, for children under the age of 6 who have difficulty brushing teeth, it is more convenient to use mouthwash than fluoride-containing toothpaste, so many children use it [24]. However, mouthwash also needs to be checked because it can be inadvertently swallowed or fluoride may remain in the mouth.

Various methods can analyze the residual amount of fluoride in the oral cavity, and among them, the analysis using ^{19}F NMR is being actively conducted. Lee *et al.* [25] reported that NMR was effective in analyzing the three-dimensional structure of bone, and Park *et al.* [26] analyzed the properties of fluorine-containing epoxy resins through ^{13}C NMR and ^{19}F NMR analysis. In addition, Stamboulis *et al.* [27] confirmed the change in the crystal structure according to the chemical reaction that may occur during adding fluoride to the glass ionomer cement. White *et al.* [28] confirmed the applicability of NMR by detecting fluorapatite through MAS-NMR when tooth powder treated with fluoride-containing toothpaste. There-

fore, in this study, NMR analyzed the components of three types of fluoride-containing mouthwashes for children. As a result, the fluoride content of 2080 Kids was 90 ppm, a low concentration, but detected TF was an average of 25.956, about 29 % less. The fluoride content indicated in Chikachika was 226 ppm, but the average TF was 153.879, which was detected about 32 % less. On the other hand, the detected TF of Garglin Kids Care marked as 226.1 ppm was with an average of 455.818, which was 102 % more. All three types of fluorine-containing mouthwashes for children on the market were different from the standards indicated by the manufacturers. Therefore, additional research is needed to confirm the fluoride standards for commercially available mouthwash through various methods of measuring fluoride.

The anti-caries effect of fluoride points out the importance of fluoride in the liquid environment around the teeth, and the concentration of fluoride in saliva indicates fluoride in the aqueous phase that can interact with the tooth surface for a given time [29]. Heintze and Petersson [30] reported that after using 0.05 % and 0.2 % sodium fluoride (NaF) mouthwash for 3 minutes, it took 2 hours and 30 minutes and 6 hours and 30 minutes respectively until the initial concentration. Bruun *et al.* [31] reported that a high concentration was maintained for up to 5 hours when keeping 10 ml of 0.2 % NaF mouthwash for 2 minutes. These reports indicate the excellent dental caries prevention effect of mouthwash. Unlike toothpaste, mouthwash containing fluoride is often not rinsed with water after application, so the residual amount of fluoride in the mouth will be higher. This residual amount of fluoride prevents dental caries, but children under the age of 6 who cannot wash their mouths on their own may have a chronic fluoride poisoning problem due to swallowing issues. Accordingly, this study measured the fluoride remaining in saliva after using fluoride-containing mouthwashes. After 1 minute of using mouthwashes, the residual fluoride was 0.0144 % for 2080 Kids, 15.4477 % for Chikachika, and 0.0015 % for Garglin Kids Care. After 2 minutes of using each mouthwash, the residual amount was 0.0068 % for 2080 Kids, 0.0013 % for Chikachika, and 0.0004 % for Garglin Kids Care, confirming very little amount is present in the oral saliva. Saliva in the oral cavity contains various minerals, and the fluoride content is about 0.1 ppm, which is similar to that of plasma [32]. Shannon [33] stated that the fluoride concentration in saliva was about 1 $\mu\text{mol/L}$, and Duckworth *et al.* [34] reported that the average fluoride concentration in mixed saliva was $0.4 \pm 0.005 \mu\text{mol/L}$ in a study involving seven adults. Bruun *et al.* [31] reported 0.02-0.05 ppm, while Heintze and Petersson [30] stated that the normal

fluoride concentration in saliva was 0.01-0.03 ppm.

Therefore, this study confirmed the mouthwash has no effect on the human body as toxicity even when children swallow the fluoride remained in the saliva after using fluoride-containing mouthwash for children. The limitations of this study are that it did not compare various commercially available oral gargles and did not classify them by age, which could be affected by the individual oral environment. Nevertheless, for children under the age of 6 who have difficulty brushing their teeth, mouthwash containing fluoride can prevent dental caries and contribute to oral health.

5. Conclusion

The residual amount of fluoride in saliva in the oral cavity was analyzed using NMR to confirm the safety of using fluoride-containing mouthwash for children under six years of age. After 2 minutes of using three types of mouthwash for children containing fluoride, only a very small amount of fluoride remained. So, there was no possibility of exposure to side effects. Therefore, fluoride-containing mouthwash is a safe method of preventing dental caries for children under six who have difficulty brushing teeth.

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