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Original Article

Dentistry Section

# Staining Effect of Amflor, Zerosense and Ceti-F Mouthwashes on Dental Enamel: In-vitro Study

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## ABSTRACT

**Introduction:** Chemical methods of plaque control, especially mouthwashes, offer great help in maintaining good oral hygiene and preventing dental caries. However, these mouthwashes often result in staining of the enamel.

**Aim:** To evaluate discolouration caused by three different fluoride mouthwashes on dental enamel.

**Materials and Methods:** This was an in-vitro study that included 40 freshly extracted premolar teeth and was conducted at University of Saveetha in January 2021. A total of four groups were made with 10 teeth in each group. Group 1 included teeth as the control group where distilled water was used, Group 2: Amflor mouthwash group, Group 3: Ceti-F (Cetyl Pyridinium Chloride, Triclosan and Sodium Fluoride) mouthwash group and Group 4: Zerosense mouthwash group. Pretreatment colour change examination according to CIELAB {International Commission on Illumination L: value or degree of lightness, a: positions on red/green (+a=red, -a=green); b: yellow/blue (+b=yellow, -b=blue) axes} colour space system was done before immersing in the mouthwash solution (T1). Then each group of teeth were immersed in their respective mouthwash for 24 hours and postimmersion colour change was determined (T2). Analysis of Variance (ANOVA) test was done to determine intergroup comparison of all four groups in terms of differences in colour changes between T1 and T2. Intragroup comparison using paired t-test was done to find out colour differences within the study groups.

**Results:** On observing total sample of 40 freshly extracted premolar teeth (10 teeth in each group. Group 1-control group, Group 2-Amflor mouthwash group, Group 3-Ceti-F mouthwash group and Group 4-Zerosense mouthwash group. ANOVA test revealed a statistically significant difference between T1 and T2 for the three mouth washes ($p<0.05$). Paired t-test for intragroup comparison revealed a statistically significant colour difference in all the three mouthwash groups except for the control group ($p<0.05$). The colour change ($\Delta E$) value was significantly higher for the Amflor group, and least for the Zerosense group. An obvious colour change was noted for all the mouthwash under study.

**Conclusion:** Fluoride mouthwashes produce an obvious staining effect on dental enamel. Among the three test groups included, Zerosense showed the least colour change.


**Keywords:** Caries prevention, Chemical plaque control, Discolouration, Fluoride mouthwashes, Remineralising agents, White spot lesion

## INTRODUCTION

There are various methods of controlling plaque retention, of which, tooth brushing and interproximal cleaning are the most efficient methods. Mouthwashes act as great supplementation for patients undergoing fixed orthodontic treatment. However, some mouthwashes have undesired effects such as staining of enamel surface, calculus formation etc. which has limited their usage among the patients. The discolouration of tooth and restorative materials by chlorhexidine has been demonstrated in several studies [1,2]. Fluoride containing mouthwashes with enhanced mechanical properties and antibacterial and anticariogenic effects are commonly advised by orthodontists as they help to counteract White Spot Lesions (WSL) and help in enamel remineralisation [3]. Earlier studies on fluoride mouthwashes focused on the effects of fluoride mouthwashes on controlling microbial colonisation, frictional resistance of different types of brackets and orthodontic wires when subjected to fluoride mouthwashes [1,4,5], effect on WSL etc., [6]. However, staining of enamel caused by different fluorides containing mouthwashes has not been studied so far except for stannous fluoride [7]. Hence, there is little information regarding the staining effect of fluoride mouthwashes on dental enamel.

Three commonly prescribed fluoride mouthwashes namely Ceti-F, Zerosense and Amflor mouthwashes were evaluated in this study. Ceti-F is a Cetylpyridinium Chloride (CPC) based mouthwash incorporated with fluoride delivering an anticariogenic and antibacterial effect. It has been shown to be effective in preventing dental plaque and reducing gingivitis [8-10]. The manufacturer claims that Ceti-F causes mild brown staining. Zerosense is primarily used to prevent dentin hypersensitivity and dental caries. It is composed of sodium fluoride and potassium nitrate. This medication is used to prevent cavities and to reduce pain from sensitive teeth (dentinal hypersensitivity). Sodium fluoride works by making the teeth more resistant to decay caused by acid and bacteria while potassium nitrate helps to reduce hypersensitivity. Amflor mouthwash contains an amine fluoride with a slightly acidic pH. This allows the fluoride ions to combine rapidly with the calcium in dental enamel to form calcium fluoride which acts as a fluoride depot on the tooth surface over a longer period. Under cariogenic conditions, the fluoride ions are made available that stimulates the remineralisation of dental enamel and thus prevent acid attacks [11]. Although these commonly used mouthwashes could greatly help in preventing WSL and are effective in plaque removal, their effects of staining of dental enamel need to be studied so as to not cause any undesired effects on the enamel when they are prescribed for long term use.

Hence, the present investigation aimed to evaluate the staining effects of Amine fluoride (Amflor), Ceti-F and sodium fluoride-potassium nitrate commonly used fluoride mouthwashes on dental enamel.

## MATERIALS AND METHODS

This in-vitro study was conducted in January 2021 at Saveetha Dental College and ethical approval was obtained from the scientific review board of Saveetha University (Approval No. IHEC/SDC/

ORTHO-2001/21/215). A sample size of 40 freshly extracted premolar teeth were grouped into four groups with ten samples in each group.

**Inclusion criteria:** Premolar teeth that were freshly extracted for orthodontic reasons without any structural defects were included in this study.

**Exclusion criteria:** Teeth with structural defects, the ones with intrinsic staining, dental caries, restorations, WSL and teeth with deep pits and fissures were excluded from the study.

Three commercially available fluoride mouthwashes namely Amflor, Ceti-F and Zerosense were studied for their tendency to stain enamel. Amine fluoride (Amflor), Ceti-F and sodium fluoride-potassium nitrate (Zerosense) are the active ingredients in these mouthwashes. The sample consisted of four groups as follows:

Group 1: Distilled water (control Group),

Group 2: Amflor mouthwash group,

Group 3: Ceti-F mouthwash group and

Group 4: Zerosense mouthwash group.

### Study Procedure

All the teeth were cleaned with water slurry of pumice using rubber prophylactic cups and were then rinsed with tap water to remove any debris. They were immersed for 24 hours in distilled water at 37°C. Following which colourimetric values were recorded (T1) using a spectrophotometer (VITA easyshade Advance) [Table/Fig-1]. The middle third of buccal surfaces of all the teeth were used to determine the colour change according to CIELAB (International Commission on Illumination L: value or degree of lightness, a: positions on red/green (+a=red, -a=green) ; b: yellow/blue (+b=yellow, -b=blue) axes) [Table/Fig-2] [12]. The teeth were then immersed in their fluoride mouthwash solutions for 24 hours during which the solutions were shaken every 3 hours to create homogeneity. The teeth were then rinsed with water for one minute, dried with cotton rolls, and colour determination was done (T2). The L, a, and b values were determined for each specimen. In this system, the L coordinate refers to the value or degree of lightness, whereas the a and b values indicate positions on red/green (+a=red, -a=green) and yellow/blue (+b=yellow, -b=blue) axes, respectively [13,14]. The colour change (ΔE) between the different treatment stages was calculated using the following formula: $\Delta E=\{(\Delta a)^2+(\Delta b)^2+(\Delta L)^2\}$ 0.5 [15].

**[Table/Fig-1]:** Samples were labelled and grouped into four; **[Table/Fig-2]:** VITA easy shade advance is being placed on the completely dried buccal surface of the teeth to determine the colour change. (Images from left to right)

Colour difference determined using ΔE values are as follows [16]:

0 <ΔE<1- observer does not notice the difference

1 <ΔE<2- only experienced observer can notice the difference

2 <ΔE<3.5- inexperienced observer also notices the difference

3.5 <ΔE<5- clear difference in colour is noticed

5 <ΔE- observer notices two different colours

All measurements were made by a single investigator experienced with vita easy shade advance, following the manufacturer's instructions.

## STATISTICAL ANALYSIS

One way analysis of variance (ANOVA) was used to compare the intergroup colour change (ΔE) between the measurements at T1 and T2 time points between the study groups. Paired t-test was done for an intragroup comparison among the groups between T1 and T2 time points. The statistical analysis was performed using Statistical Package for the Social Sciences (version 23.0, SPSS Inc, Chicago, Ill) and the significance level was determined at $p<0.05$.

## RESULTS

A total of 40 samples of extracted teeth were observed. The values obtained from VITA easy shade Advance are displayed in [Table/Fig-3]. Intergroup comparison using ANOVA test revealed significant colour change in all the three test groups at time intervals of 24 hours (T2) when colour change was determined against the control group

| Groups | L | | a | | b | | delta E (ΔE) | Mean colour change | Inference |
|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T1 | T2 | T1 | T2 | | | |
| Control | 85.3 | 85.3 | 3.5 | 3.5 | 20 | 20 | 0 | 0.1 | Observer does not notice the difference |
| | 83.2 | 83 | 1.7 | 1.7 | 16.2 | 16.3 | 0.2 | | |
| | 85.8 | 85.6 | 2.1 | 2.1 | 17.3 | 17.4 | 0.2 | | |
| | 91.6 | 91.6 | 2.2 | 2.2 | 17.5 | 17.4 | 0.1 | | |
| | 86 | 85.9 | 1.7 | 1.7 | 20.2 | 20.2 | 0.1 | | |
| | 94.8 | 94.7 | 0.1 | 0.2 | 17 | 17 | 0.14 | | |
| | 88.8 | 88.8 | 0.8 | 0.8 | 13.1 | 13.1 | 0 | | |
| | 90.4 | 90.3 | 3.2 | 3.2 | 24.9 | 24.7 | 0.2 | | |
| | 85.9 | 85.9 | 3.3 | 3.3 | 20.5 | 20.5 | 0.1 | | |
| | 69.6 | 69.6 | 6 | 6 | 24 | 24 | 0 | | |
| Amflor | 80.4 | 80.4 | 5.2 | 8.6 | 29.5 | 35.1 | 6.5 | 6.1 | A very obvious difference, observer notices two different colours |
| | 77.9 | 76.5 | 2.7 | 2.5 | 16.2 | 16.5 | 7.9 | | |
| | 84.4 | 87.3 | 1.5 | 3.1 | 15.2 | 21.8 | 7.3 | | |
| | 82.8 | 87.4 | 0.8 | 0.8 | 15.2 | 18.5 | 5.6 | | |
| | 84.5 | 91.3 | 3.1 | 3.8 | 17.2 | 22.9 | 8.9 | | |
| | 86.9 | 89.3 | 2.7 | 2.3 | 19.8 | 24 | 4.8 | | |
| | 84.7 | 87.1 | 3.3 | 4 | 22.4 | 27.7 | 5.8 | | |
| | 84.4 | 88.5 | 0.9 | 1.4 | 16.3 | 21.6 | 6.7 | | |
| | 91.1 | 92 | 2.4 | 2.9 | 18.1 | 21.1 | 3.1 | | |
| | 84.5 | 84.9 | 1.1 | 1.4 | 14.4 | 19.1 | 4.7 | | |
| Ceti-F | 85.7 | 89 | 2.1 | 3.4 | 18.1 | 25.1 | 7.8 | 5.8 | A very obvious difference, observer notices two different colours |
| | 74.1 | 77 | 3.5 | 4.3 | 19.2 | 22.9 | 4.7 | | |
| | 81.8 | 84.5 | 2.7 | 2.6 | 20.1 | 24.1 | 4.8 | | |
| | 82.1 | 83.2 | 3.7 | 3 | 22.6 | 25.7 | 3.3 | | |
| | 80.5 | 81.1 | 3.8 | 4.9 | 18.1 | 24.6 | 6.6 | | |
| | 56.6 | 60.5 | 2.8 | 6.4 | 12.9 | 19.8 | 8.7 | | |
| | 78.9 | 78.7 | 3.8 | 4.9 | 22.1 | 26 | 4 | | |
| | 93.5 | 91.4 | 1.4 | 6.8 | 18.7 | 21.8 | 6.5 | | |
| | 88.9 | 91.7 | 3.3 | 4.6 | 21.9 | 28.8 | 7.5 | | |
| | 96.7 | 97.4 | 0.8 | 0.7 | 11.9 | 16.1 | 4.2 | | |
| Zerosense | 88.5 | 88.7 | 1.9 | 1.6 | 14.2 | 17 | 2.8 | 3.8 | Obvious difference, clear difference in colour is noticed |
| | 88.8 | 94.3 | 2 | 1.7 | 16.2 | 20.6 | 7 | | |
| | 81.6 | 86.1 | 3.4 | 2.5 | 22 | 24.8 | 5.3 | | |
| | 75.2 | 73.9 | 1.2 | -0.1 | 10.4 | 12.2 | 2.4 | | |
| | 81 | 79.9 | 1.7 | 1.2 | 22 | 24.6 | 2.8 | | |
| | 74.7 | 75 | 4.3 | 1.7 | 20.6 | 20.9 | 2.6 | | |
| | 91.1 | 92.3 | 1.9 | 1.1 | 15 | 16.8 | 2.3 | | |
| | 83.9 | 89.7 | 2.4 | 1.5 | 21.5 | 27 | 8 | | |
| | 84.4 | 88.4 | 1.9 | 0.8 | 18.7 | 21.3 | 4.8 | | |
| | 80.7 | 80.7 | 2.5 | 1.8 | 19.4 | 19.3 | 0.7 | | |

**[Table/Fig-3]:** Mean delta-E values of three groups included in the study.
ΔE: Colour change measurement; the L coordinate refers to the value or degree of lightness, the a and b values indicate positions on red/green (+a=red, -a=green) and yellow/blue (+b=yellow, -b=blue) axes, respectively. (CIELAB)

[Table/Fig-4]. Significant differences were also noted between Zerosense and Amflor group (p<0.05). The greatest colour change was observed in the Amflor group (very obvious difference) and least in the Zerosense group (obvious difference). Intragroup comparison revealed a statistically significant colour change in all the test groups (p<0.05). Mean and standard deviation of colour change observed in all three groups are shown in [Table/Fig-5]. Paired t-test was done to find out the intragroup comparison [Table/Fig-6].

| (I) Mouthwash | (J) Mouthwash | Mean difference (I)-(J) | Std. Error | p-value |
|---|---|---|---|---|
| Control group | Amflor | -6.02600 | 0.76417 | **<0.001*** |
| | Ceti-F | -5.70600 | 0.76417 | **<0.001*** |
| | Zerosense | -3.76600 | 0.76417 | **<0.001*** |
| Amflor | Control group | 6.02600 | 0.76417 | **<0.001*** |
| | Ceti-F | 0.32000 | 0.76417 | 0.975 |
| | Zerosense | 2.26000 | 0.76417 | **0.027*** |
| Ceti-F | Control group | 5.70600 | 0.76417 | **<0.001*** |
| | Amflor | -0.32000 | 0.76417 | 0.975 |
| | Zerosense | 1.94000 | 0.76417 | 0.071 |
| Zerosense | Control group | 3.76600 | 0.76417 | **<0.001*** |
| | Amflor | -2.26000 | 0.76417 | **0.027*** |
| | Ceti-F | -1.94000 | 0.76417 | 0.071 |

**[Table/Fig-4]:** ANOVA test showing intergroup comparison among mouthwashes depicting significant colour change in all the three intervention group (p<0.05).
*Significant

| Mouthwash groups | N | Mean | Std. Deviation |
|---|---|---|---|
| Control group | 10 | 0.10 | 0.083 |
| Amflor | 10 | 6.13 | 1.700 |
| Ceti-F | 10 | 5.81 | 1.847 |
| Zerosense | 10 | 3.87 | 2.318 |

**[Table/Fig-5]:** Descriptive statistics showing mean and SD colour change of the four groups.

| Groups | T1-T2 | Significance |
|---|---|---|
| Group 1 (Control) | L | 0.250 |
| | a | 0.343 |
| | b | 0.726 |
| Group 2 (Amflor) | L | 0.015* |
| | a | 0.071 |
| | b | <0.001* |
| Group 3 (Ceti-F) | L | 0.026* |
| | a | 0.043* |
| | b | <0.001* |
| Group 4 (Zerosense) | L | 0.050* |
| | a | 0.002* |
| | b | 0.001* |

**[Table/Fig-6]:** Paired t-test depicting intragroup comparison of colour change for four groups between T1 and T2.
Paired t-test; *Significant

## DISCUSSION

Mechanical methods of plaque control although provide efficient cleaning, it does have some difficulties when it comes to surgically treated patients or orthodontic patients where effective cleaning is hindered by the appliance resulting in WSL etc. In these conditions, mouthwashes play a larger role in plaque control, which, however can lead to staining of teeth. To overcome this, mouthwashes with the least staining effect should be prescribed to patients. One study on chlorhexidine mouthwash has evaluated the microbial control and staining effects of nanoparticle infused chlorhexidine [1]. The present study focussed on the effect of discolouration of fluoride mouthwashes. All the three mouthwashes included in the study showed significant colour change of dental enamel after 24 hours of immersion. The results of the study suggest that staining of enamel is least with Zerosense mouthwash compared with Amflor and Ceti-F groups. Staining effect of Amine fluoride was reported in a previous study [11] and similar results were found in this study.

Varied chemical composition of these mouthwashes makes it unclear if the staining of dental enamel is due to fluoride or the other chemical used in the mouthwash. However, fluoride being included in greater percentages in these mouthwashes, staining of dental enamel is considered majorly of fluoride. Also, the pretreatment colour determination values (T1) were almost the same in the control group postimmersion (T2). Statistically significant difference in terms of colour change was found for all three (Amflor, Ceti-F, Zerosense) mouthwashes (p<0.05) of which Zerosense showed the least colour change followed by Ceti-F and Amflor. Paired t-test showed significant colour changes observed in all the three test groups between T1 and T2.

Fluoride mouthwashes are prescribed commonly to improve oral hygiene, to prevent WSL and cause remineralisation. Orthodontists mostly recommend a daily 0.05 percent sodium fluoride rinse in conjunction with fluoridated toothpaste as the most common oral hygiene protocol [17]. However, while this recommendation is based on the fact that sodium fluoride rinse reduces dental caries rates in non orthodontic patients, the evidence for its effectiveness in preventing WSL in orthodontic patients is mixed [17]. An article by Mekki A et al., has reported that fluoride varnish applied every six weeks during orthodontic treatment is effective [3].

Very little information on staining effects of fluoride mouthwashes is available in the literature. The results of this study have shown that fluoride mouthwashes have caused significant staining of dental enamel. There are various methods of assessing the colour change of enamel [18]. These methods of colour assessment help us in acknowledging the accurate colour change which is difficult to ascertain with the naked eye. Here, a spectrophotometer (VITA easy shade Advance) was used to determine colour changes in dental enamel after being immersed in fluoride mouthwashes. The device express tooth shades in a shade guide system, using conversion scales for CIE L*a*b* values, based on internally stored data [16]. This function is performed automatically.

Eslami N et al., reported staining effects of chlorhexidine and mouthwash containing nanoparticles and concluded nanoparticle infused mouthwashes produced greater stain than chlorhexidine [1]. Earlier studies involving fluoride mouthwashes have evaluated the antimicrobial activity of fluoride mouthwashes and its efficacy in controlling plaque formation [11,19,20] and fluoride uptake by enamel and dentine and also its substantivity etc., [11,19-28]. None of these studies reported information on staining of dental enamel caused by fluoride mouthwashes. In this study, all the mouthwashes included have greater colour change when compared to the control group. Of which Zerosense has significantly lesser colour change. The ANOVA test showing intergroup comparison reveals statistically significant differences (p<0.05) are noted between Amflor and Zerosense. Caution should be taken to prescribe mouthwashes to patients on the long-term considering its staining effect. Mouthwashes with less severe staining effects have to be prescribed. A study by Frese C et al., on clinical effectiveness of stannous fluoride and amine fluoride reported significant staining with fluoride mouthwashes when compared with a non treatment control group which correlates with the current study [29].

### Limitation(s)

The limitations of the study include a smaller sample size, use of concentrated mouthwashes, longer duration of immersion. This could have caused greater staining of teeth with the mouthwashes. Future studies should overcome these limitations and provide stable results.

## CONCLUSION(S)

Within the limitations of the study, all fluoride mouthwashes (Amflor, Ceti-F, Zerosense) produced significant staining of dental enamel. Among the three groups included, Zerosense showed the least colour change followed by Ceti-F and Amflor. Intergroup comparison revealed significant colour change between control group and all three mouthwash groups (Amflor, Ceti-F, Zerosense) and between Amflor and Zerosense group. Intragroup comparison revealed significant colour change in all the three test groups.

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